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Cyclooxygenase 2 expression by endothelin-1-stimulated mouse resident peritoneal macrophages in vitro

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Abstract

Macrophages have been shown to produce endothelin and to play a role in the pathogenesis of neural damage after cerebral ischemia or vasospasm after subarachnoid hemorrhage. Cyclooxygenase 2 is induced during inflammation following brain insult and participates in inflammation-mediated neurotoxicity. However, it has not yet been established how endothelin-1 acts on cyclooxygenase 2 expression in macrophages. In the present study, we examined the effects of endothelin-1 on cyclooxygenase 2 expression and prostaglandin E_2 production, and the effects of endothelin ET_A and ET_B receptor antagonists. Stimulation by endothelin-1 ranging from 10^{-11} to 10^{-9} M time and dose dependently increased the production of prostaglandin E_2 and the expression of cyclooxygenase 2 protein without changing that of cyclooxygenase 1 protein, an effect which was inhibited by dexamethasone, nonsteroidal anti-inflammatory drugs and the selective endothelin ET_B receptor antagonist, BQ788 (*N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methyl-leucyl-D-L-methoxycarbonyl-tryptophanyl-D-norleucine). The selective endothelin ET_A receptor antagonist, BQ123 [cyclo (D-Trp-D-Asp-Pro-D-Val-Leu)] also inhibited these reactions, but its potency was less than that of the selective endothelin ET_B receptor antagonist. Endothelin ET_A and ET_B receptor antagonists had no effects on cyclooxygenase 2 protein expression and prostaglandin E_2 production in lipopolysaccharide-stimulated macrophages. We conclude that endothelin-1 increases cyclooxygenase 2 protein expression and prostaglandin E_2 production via mainly endothelin ET_B receptors and partly endothelin ET_A receptors in macrophages; however, lipopolysaccharide increases both cyclooxygenase 2 protein expression and prostaglandin E_2 production in pacrophages without involving endothelin ET_A or ET_B receptor-mediated processes.

Keywords: Endothelin-1; Macrophage; Cyclooxygenase 2; Endothelin ET_A receptor antagonist; Endothelin ET_B receptor antagonist

1. Introduction

Endothelin-1 is produced by macrophages, endothelial cells and astrocytes, and is the most potent and long lasting vasoconstrictive peptide identified to date (Yanagisawa et al., 1988; Nie and Olsson, 1996). It has been recognized to play an important role in the pathogenesis of neural damage after cerebral ischemia (Juzan et al., 1992; Kochanek and Hallenbeck, 1992; Yamashita et al., 1994) and vasospasm after subarachnoid hemorrhage (Yamaura et al., 1992; Kobayashi et al., 1995; Kita et al., 1998). Macrophages have been shown to infiltrate into injured neural tissue (Fischer and Bogousslavsky, 1996) and are also detected during cerebral ischemia and vasospasm after subarachnoid hemorrhage (Merrill and Benveniste, 1996). Inflammation-mediated cytotoxicity recently has emerged as one of the important factors that may play a role in the delayed progression of neural damage following several types of brain insult. Moreover, cyclooxygenase 2 is expressed in inflammatory cells such as macrophages after stimulation with endotoxin, cytokines, etc., and its reaction products are responsible for many cytotoxic effect of inflammation.

This study was designed to evaluate the role of endothelin-1 using mouse resident peritoneal macrophages. The expression of the expression of the rate-limiting enzyme for prostanoids, cyclooxygenase 2, and the production of prostaglandin E_2 in macrophages were determined as markers for evaluating the effect of endothelin-1 stimula-

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tion. We also compared the effects of exogenously given endothelin-1 with those elicited under inflammatory conditions obtained by using lipopolysaccharide.

Dexamethasone, nonsteroidal anti-inflammatory drugs and endothelin receptor antagonists were used to investigate the difference in the effects of the stimulations.

2. Materials and methods

2.1. Cell culture

This study was performed in accordance with The Japanese Pharmacological Society Guide for the Care and Use of Laboratory Animals. All procedures. All procedures were approved by the local Animal Care Committee at Nara Medical University.

Resident peritoneal macrophages were collected by lavage of the peritoneal cavity of 10-week-old ddY mice (Kiwa Experimental Laboratory Animal, Wakayama, Japan) with 5.0 ml of sterile heparinized calcium and magnesium-free phosphate buffer saline (PBS). The cells collected were immediately centrifuged at 1000 rpm for 10 min at 4°C. The supernatants were discarded and peritoneal cells were immediately seeded in 12-well plates in 1 ml of Dulbecco's modified Eagle's medium (DMEM; Gibco-BRL, France) containing 3% fetal bovine serum (Nacalai Tesque, Kyoto, Japan). After a 90-min incubation at 37°C with 5% CO_2, almost all adherent cells were resident peritoneal macrophages, as assessed by measurement of their esterase activity. Then the cells were suspended at 1.0×10^6 cells/ml in DMEM and seeded in 12-well plates (1 ml/well). Cell viability through the experiment was about 95% with trypan blue dye exclusion and measurement of lactate dehydrogenase (LDH) in cell supernatants with an LDH–UV test kit (Wako, Tokyo, Japan).

Adherent macrophages were washed with sterile calcium and magnesium-free PBS warmed to 37°C and incubated in the medium with or without endothelin-1 (Peptide Institute, Kyoto, Japan) ranging from 10^{-12} to 10^{-8} M for up to 24 h or with 10 μg/ml lipopolysaccharide (*Escherichia coli*, 055: B5, Nacalai Tesque) at 37°C with 5% CO_2.

In some experiments, the cells were pretreated with dexamethasone (Nacalai Tesque), nonsteroidal anti-inflammatory drugs [indomethacin, NS398 (Cayman, MI, USA)] and endothelin receptor antagonists, and then stimulated by endothelin-1 or lipopolysaccharide to estimate their inhibitory effects on cyclooxygenase 2 protein expression and prostaglandin E_2 production in macrophages.

2.2. Western blot

Macrophage proteins (40 μg) were separated by electrophoresis on 7.5% sodium dodecyl sulfate-polyacrylmide gels and transferred to hybond polyvinylidene difluoride membranes (Amersham, Tokyo, Japan). Cyclooxygenase 1 and 2 were detected with rabbit polyclonal antiserum against cyclooxygenase 1 and 2 or against cyclooxygenase 2 (diluted 1:1000; Cayman) and goat anti-rabbit immunoglobulin G-horseradish peroxidase (diluted 1:1000; Amersham).

Peroxidase activity was visualized by the enhanced chemiluminescence detection system (Amersham) using Kodak X-AR film. Determination of cyclooxygenase 1 and 2 was performed by densitometry with a densitometer (Bio-Rad Laboratories, Tokyo, Japan).

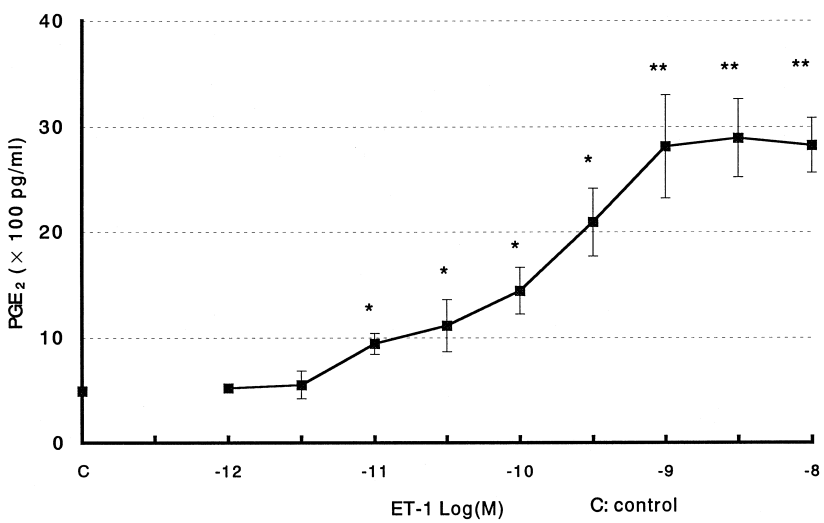

Fig. 1. Dose-dependent production of prostaglandin E_2 by macrophages stimulated by endothelin-1. Mouse peritoneal macrophages were collected in Dulbecco's modified Eagle's medium. After the cells had been allowed to adhere for 90 min, they were incubated with endothelin-1 for 12 h. Endothelin-1 was tested at concentrations ranging from 10^{-12} to 10^{-8} M. For each experiment, prostaglandin E_2 level in supernatants obtained from each well was measured by enzyme immunoassay. Values are expressed as the means ± S.D. of eight independent experiments. $* P < 0.05$ and $** P < 0.01$ indicate statistically significant differences from the corresponding values for saline treatment (control).

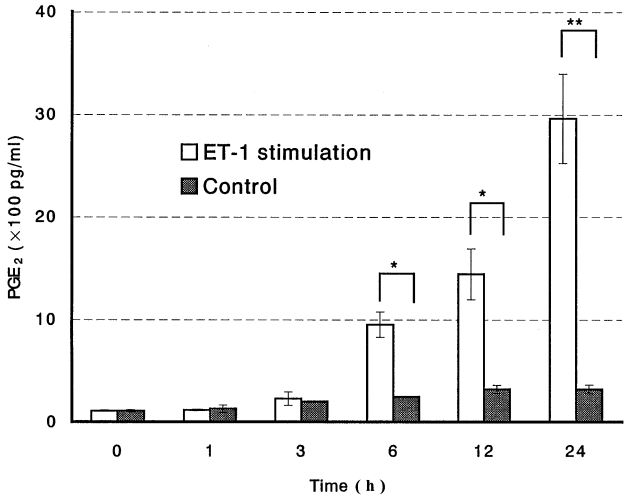

Fig. 2. Time-dependent production of prostaglandin E_2 by macrophages stimulated by endothelin-1. Mouse peritoneal macrophages were collected in Dulbecco's modified Eagle's medium. After the cells had been allowed to adhere for 90 min, they were incubated with 10^{-10} M of endothelin-1 for different times (0, 1, 3, 6, 12, and 24 h). For each experiments, prostaglandin E_2 level in supernatants obtained from each well was measured by enzyme immunoassay. Values are expressed as the means ± S.D. of eight independent experiments. $*P < 0.05$ and $**P < 0.01$ indicate statistically significant differences from the corresponding values for saline treatment (control).

The specificity of the antibodies used for immunodetection of cyclooxygenase was determined in the presence of two purified forms of prostaglandin synthetase (ram seminal vesicle cyclooxygenase 1 and sheep placenta cyclooxygenase 2) purchased from Cayman; rabbit anti-human cyclooxygenase 1 and 2 antibodies diluted 1:1000 detected ram seminal vesicle cyclooxygenase 1 (70 kDa) and did not cross-react with sheep placenta cyclooxygenase 2 (78 kDa) or murine cyclooxygenase 2 (72–74 kDa). Rabbit polyclonal antiserum against human and murine cyclooxygenase 2 did not cross-react with murine cyclooxygenase 1 (68 kDa).

2.3. Method for measuring drug interactions with cyclooxygenase 2 protein expression

The effects of dexamethasone, nonsteroidal anti-inflammatory drugs and endothelin receptor antagonists on cyclooxygenase 2 protein expression were evaluated by using 10^{-10} M endothelin-1-stimulated macrophages (12 h) and 10 μg/ml lipopolysaccharide-stimulated macrophages (4 h). Drugs were added 30 min before stimulation endothelin-1 or lipopolysaccharide. Dexamethasone was tested at 10^{-6} M (Tordjman et al., 1995).

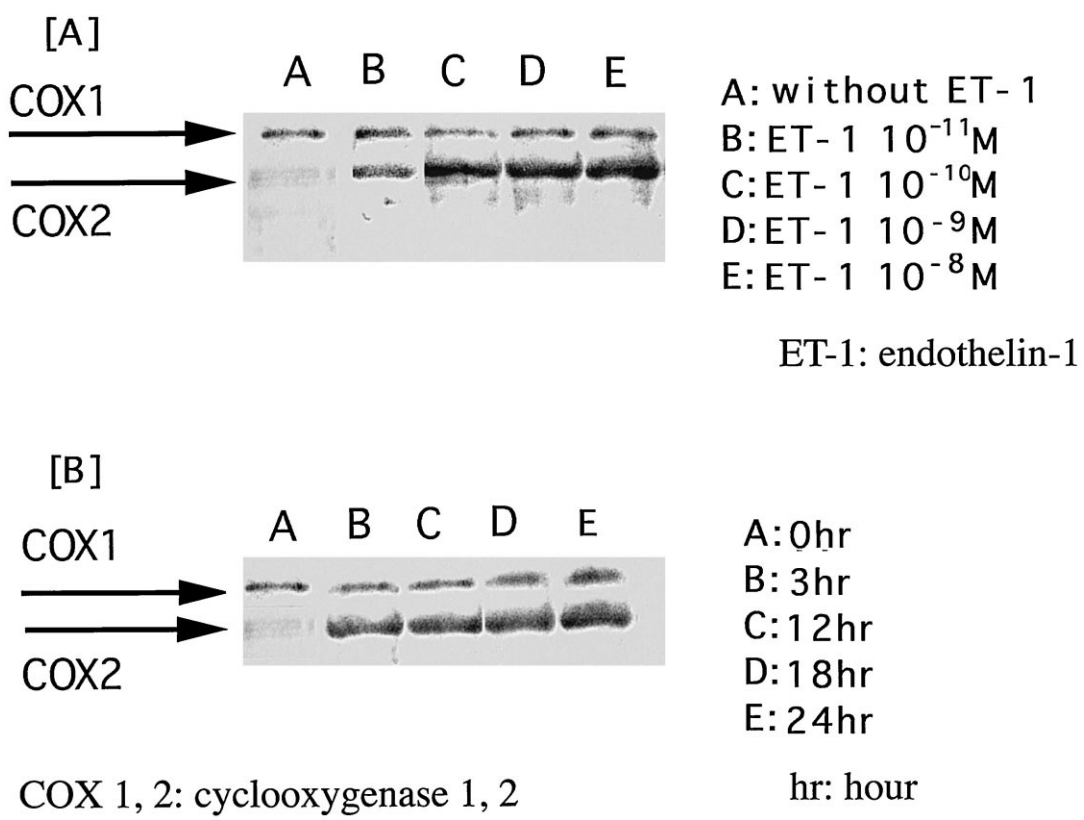

Fig. 3. Changes in cyclooxygenase 1 and 2 protein expression by macrophages stimulated by endothelin-1. Mouse peritoneal macrophages were collected in Dulbeccos's modified Eagle's medium. After the cells had been allowed to adhere for 90 min, (A) they were incubated with endothelin-1 ranging from 10^{-11} to 10^{-8} M or without it for 12 h. (B) The cells were incubated with 10^{-10} M of endothelin-1 for different times (0, 3, 12, 18 and 24 h). Macrophage proteins were collected and cyclooxygenase 1 and 2 protein expression was detected by Western blot as described in Section 2.

Indomethacin and NS398 were tested at concentrations close to their IC_{50} on cyclogenase 1, as determined with zymosan-stimulated nonadherent macrophage: 5×10^{-7} M for indomethacin and 5×10^{-5} M for NS398.

Endothelin receptor antagonists were tested at the concentration close to that which completely blocks endothelin ET_A and ET_B receptors (Mihara et al., 1994): 10^{-7} M for BQ123 [cyclo (D-Trp-D-Asp-Pro-D-Val-Leu)] and BQ788 (*N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methyl-L-leucyl-D-L-methoxycarbonyl-tryptophanyl-D-norcleucine) (Banyu, Tokyo, Japan).

2.4. LDH and prostaglandin E_2 assays

The cytotoxicity of reagents was assessed by measurement of LDH in cell supernatants, using an LDH–UV test kit (Wako). The prostaglandin E_2 level in supernatants was measured by using a prostaglandin E_2 enzyme immnuoassay kit (Cayman). We assessed whether the prostaglandin E_2 concentration in Dulbecco's modified Eagle's medium (Gibco-BRL) containing 3% fetal bovine serum without cultured macrophages changed within 24 h and verified that it was stable in the present study.

2.5. Statistical analysis

Data are expressed as the means ± standard deviation (S.D.). One-way analysis of variance (ANOVA) was used to determine group differences. If the group values were statistically significant ($P < 0.05$ or $P < 0.01$), post-hoc analyses were conducted using the Fisher's Protected Least-Significant Difference (PLSD) test. Comparison of

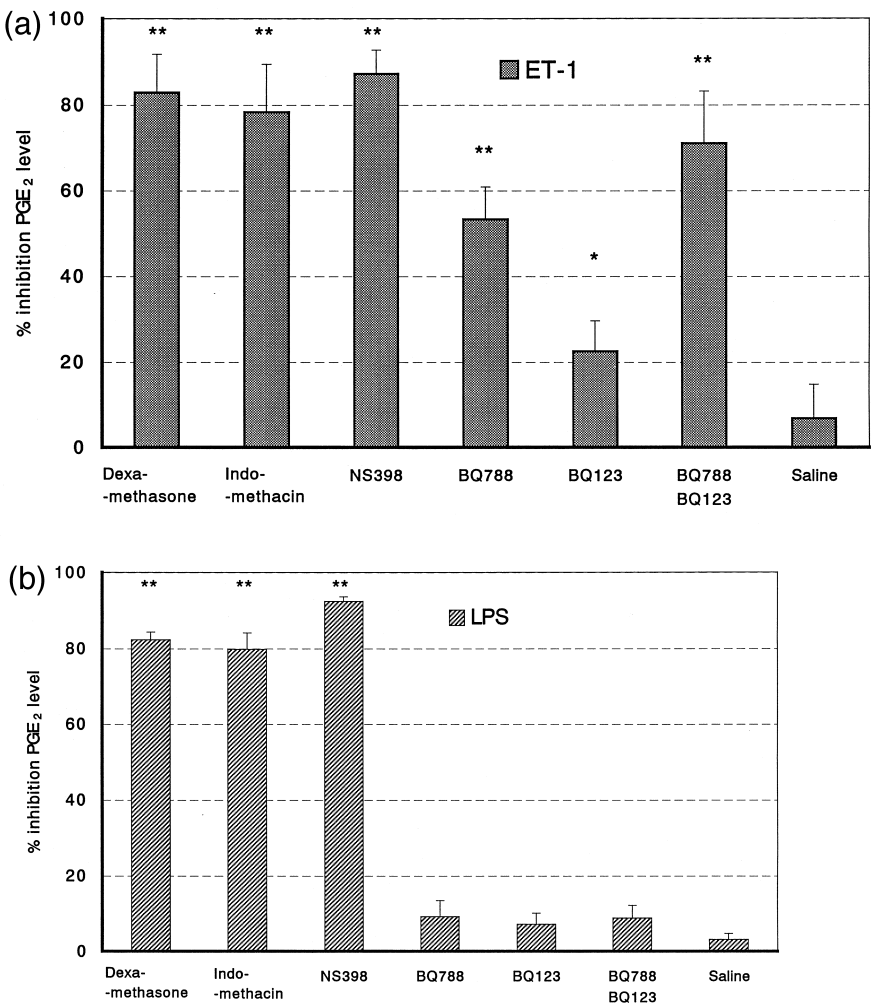

Fig. 4. Effects of dexamethasone, nonsteroidal anti-inflammatory drugs and endothelin receptor antagonists on prostaglandin E_2 production by macrophages stimulated by endothelin-1 and lipopolysaccharide. Mouse peritoneal macrophages were collected in Dulbecco's modified Eagle's medium. After the cells had been allowed to adhere for 90 min, they were preincubated with dexamenthasone, non steroidal anti-inflammatory drugs (indomethacin and NS398) and endothelin receptor antagonists for 30 min before stimulation by (a) endothelin-1 for 12 h or (b) lipopolysaccharide for 4 h. Dexamethasone and nonsteroidal anti-inflammatory drugs were tested at the following concentrations: dexamenthasone ant 10^{-6} M, indomethacin at 5×10^{-7} M, and NS398 at 5×10^{-5} M. Endothelin receptor antagonists were tested at the following concentrations: BQ788 and BQ123 at 10^{-7} M. After incubation with endothelin-1 for 12 h or lipopolysaccharide for 4 h, prostaglandin E_2 level in supernatants obtained from each well was measured by enzyme immunoassay. Values are expressed as the means ± S.D. of six independent experiments. $*P < 0.05$ and $**P < 0.01$ indicate statistically significant differences from the corresponding values for saline treatment.

two different groups was done by using the unpaired *t*-test for data presented in Fig. 2 and the one-way ANOVA with fisher's PLSD test for other data.

3. Results

3.1. Prostaglandin E_2 production in macrophages by endothelin-1

Endothelin-1 ranging from 10^{-11} to 10^{-9} M increased prostaglandin E_2 production in macrophages dose dependently. The production of prostaglandin E_2 in macrophages without endothelin-1 was 321 ± 39 and 321 ± 42 pg/ml for a 12-h and a 24-h incubation, respectively (Fig. 2). The amount measured after a 12-h incubation with 10^{-11}, 10^{-10} and 10^{-9} M of endothelin-1 was 940 ± 99, 1440 ± 221, 2810 ± 490 pg/ml, respectively (Fig. 1). This increase in prostaglandin E_2 production was not observed up to 3 h after stimulation by endothelin-1, but was clearly detected at 6 h and thereafter (Fig. 2).

3.2. Cyclooxygenase 2 protein expression by macrophages stimulated by endothelin-1

Endothelin-1 increased cyclooxygenase 2 protein expression dose and time dependently without changing cyclooxygenase 1 protein expression in mouse peritoneal

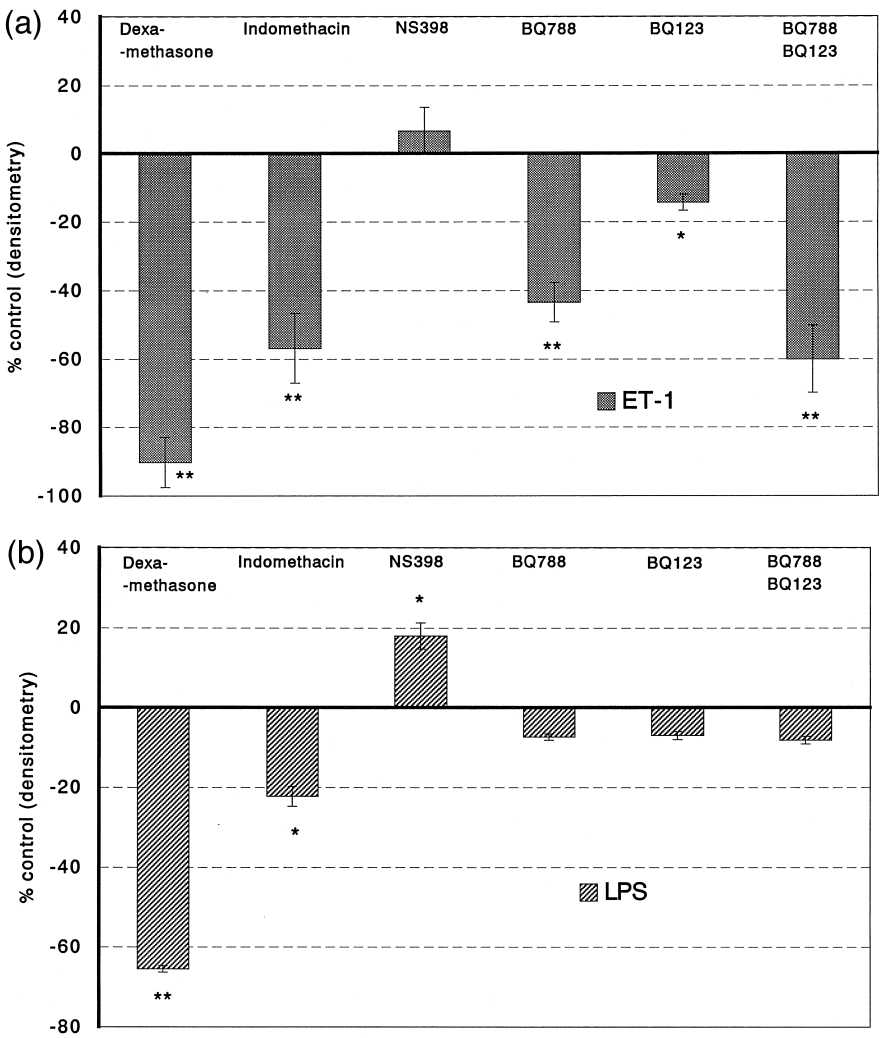

Fig. 5. Effects of dexamethasone, nonsteroidal anti-inflammatory drugs and endothelin receptor antagonist on cyclooxygenase 2 protein expression by macrophages stimulated by endothelin-1 and lipopolysaccharide. Mouse peritoneal macrophages were collected in Dulbecco's modified Eagle's medium. After the cells had been allowed to adhere for 90 min, they were preincubated with dexamethasone, non steroidal anti-inflammatory drugs (indomethacin and NS398) and endothelin receptor antagonists for 30 min before stimulation by (a) endothelin-1 for 12 h or (b) lipopolysaccharide for 4 h. Dexamethasone and nonsteroidal anti-inflammatory drugs were tested at the following concentrations: dexamethasone at 10^{-6} M, indomethacin at 5×10^{-7} M and NS398 at 5×10^{-5} M. Endothelin receptor antagonist were tested at the following concentrations: BQ788 and BQ123 at 10^{-7} M. After incubation with endothelin-1 for 12 of lipopolysaccharide for 4 h, macrophage proteins were collected and cylcooxygnase 2 protein expression was detected by Western blot as described in Materials and methods. Densitometric results of each band are expressed as percent inhibition of control. They are representative results of Western blots and the average results of the densitometric analysis of two separate experiments. $*\ P < 0.05$ and $**\ P < 0.01$ indicate statistically significant differences from the corresponding values for saline treatment.

macrophages. Endothelin-1 ranging from 10^{-11} to 10^{-8} M increased cyclooxygenase 2 protein expression in a dose-dependent manner (Fig. 3(A)). Although cyclooxygenase 2 protein expression in macrophages was detected before addition of endothelin-1, a significant increase of its expression was observed at 3 h after treatment with 10^{-10} M endothelin-1 (Fig. 3(B)). Cyclooxygenase 2 protein was detected earlier than the increase in prostaglandin E_2 production (Fig. 2). In contrast, cyclooxygenase 1 protein expression was detected in macrophages with or without endothelin-1 and its protein level was unchanged after treatment with endothelin-1 (Fig. 3(A) and (B)).

3.3. Effects of dexamethasone, nonsteroidal anti-inflammatory drugs and endothelin receptor antagonist on prostaglandin E_2 production

Dexamethasone, indomethacin and NS398 significantly inhibited prostaglandin E_2 production in endothelin-1- and lipopolysaccharide-stimulated macrophages. Both endothelin ET_A and ET_B receptor antagonists reduced prostaglandin E_2 production in endothelin-1- and lipopolysaccharide-stimulated macrophages. In addition, pretreatment with BQ788 and BQ123 together reduced prostaglandin E_2 production ($53.2 \pm 7.1\%$ for BQ788, $22.5 \pm 8.6\%$ for BQ123 and $71 \pm 12\%$ for BQ788 plus BQ123), but had no significant effects on lipopolysaccharide-stimulated macrophages (Fig. 4(a) and (b)).

3.4. Effects of dexamethasone, nonsteroidal anti-inflammatory drugs and endothelin receptor antagonists on cyclooxygenase 2 protein expression

Fig. 5 represents the results of densitometric analysis of the effects of drugs on cyclooxygenase 2 protein expression. Dexamethasone and indomethacin significantly inhibited cyclooxygenase 2 protein expression in endothelin-1- and lipopolysaccharide-stimulated macrophages. In contrast, NS398 (5×10^{-5} M) increased cyclooxygenase 2 protein expression in lipopolysaccharide-stimulated macrophages. Both endothelin ET_A and ET_B receptor antagonist reduced cyclooxygenase 2 protein expression in endothelin-1-stimulated macrophages. In addition, pretreatment with BQ788 and BQ123 together reduced cyclooxygenase 2 protein expression markedly ($43.6 \pm 58\%$ For BQ788, $14.4 \pm 2.4\%$ for BQ123 and $60.2 \pm 9.8\%$ for BQ123), but had no significant effects on cyclooxygenase 2 protein expression by lipoposaccharide-stimulated marcophages (Fig. 5(a) and (b)).

4. Discussion

There are many reports about macrophage infiltration (Kochanek and Hallenbeck, 1992; Yamashita et al., 1994; Nie and Olsson, 1996) and the role of endothelin-1 produced in macrophages in several brain diseases (Kobayashi et al., 1995; Merrill and Benveniste, 1996). However, the role of endothelin-1 on the function of macrophages has not yet been elucidated.

In the present study, we tried to evaluate the effects of exogenously given endothelin-1 on prostaglandin E_2 production in mouse resident peritoneal macrophages. Endothelin-1-induced cyclooxygenase 2 protein expression without affecting cyclooxygenase 1 protein expression, resulting in the promotion of prostaglandin E_2 production. Although the precise role of cyclooxygenase 2 in the central nervous system remains unclear, cyclooxygenase 2 has recently emerged as an important factor in the neurotoxicity seen in various brain diseases associated with inflammation (Juzan et al., 1992; Tsai et al., 1994; Chan, 1996; Nogawa et al., 1997).

First of all, an important finding of the present study is that an increase in cyclooxygenase 2 protein expression promoted prostaglandin E_2 production in endothelin-1-stimulated macrophages. There are two isoenzymes of cyclooxygenase, namely the constitutive form cyclooxygenase 1 and the inducible form cyclooxygenase 2. Cyclooxygenase 1 protein expression did not change during endothelin-1 stimulation, while cyclooxygenase 2 protein expression was increased time and dose dependently. Moreover, cyclooxygenase 2 protein expression elicited by endothelin-1 was detectable at 3 h after the start of stimulation, when prostaglandin E_2 production had not increased significantly. The cyclooxygenase 2 selective inhibitor, NS398 (Futaki et al., 1994; Hirata, 1994), almost completely inhibited prostaglandin E_2 production in endothelin-1- or lipopolysaccharide-stimulated macrophages, without causing inhibition of cyclooxygenase 2 protein expression. In addition, it has been recently demonstrated that macrophages obtained from cyclooxygenase 2 knockout mice can not produce prostaglandin E_2 when stimulated by lipopolysaccharide (Langenbach et al., 1995; Morham et al., 1995). Thus, it is presumed that the rate-limiting enzyme for prostaglandin E_2 synthesis in endothelin-1- or lipopolysaccharide-stimulated macrophages is cyclooxygenase 2, and an increase in this enzyme promotes prostaglandin E_2 production.

However, previous reports demonstrated that cyclooxygenase 2 is localized in the endoplasmic reticulum and nuclear envelope, where it converts arachidonic acid produced from membrane stores via phospholipase A_2 activation to prostanoids such as prostaglandin E_2 (Reddy and Herschman, 1994; Lu et al., 1995). Rubanyi and Polokoff (1994) showed that endothelin-1 activates phospholipase A_2 via endothelin ET_B receptors coupled to G protein in endothelial cells. In the present study, although we did not determine the change in the level of arachidonic acid or phospholipase A_2, such a mechanism of action may be partly involved in the process of prostaglandin E_2 production in endothelin-1-stimulated macrophages. However, the main mechanism is the promotion of cyclooxygenase 2

protein expression via endothelin ET_A and ET_B receptor-mediated processes, because the extent of inhibition of cyclooxygenase 2 protein expression and prostaglandin E_2 production was similar for both receptor antagonists, and the effect of combination treatment was additive. The inhibition of prostaglandin E_2 production was always slightly greater than the inhibition of cyclooxygenase 2 protein expression, so there is probably another minor inhibitory mechanism. The combination of BQ123 and BQ788 (both 10^{-7} M) was tested for inhibition of cyclooxygenase 2 protein expression by endothelin-1 (10^{-11} or 10^{-10} M)-stimulated macrophages. This combination inhibited endothelin-induced cyclooxygenase 2 protein expression by more than 60%, but complete inhibition could not be achieved, Thus other minor mechanisms besides BQ123- and BQ788-sensitive endothelin receptor-mediated processes may be involved in macrophages.

Yamashita et al. (1997) have already demonstrated a functional endothelin ET_B receptor in rat peritoneal macrophages. However, our results for mouse resident peritoneal macrophages showed that endothelin ET_A and ET_B receptor-mediated processes exist, although we have no direct evidence for the expression of the two receptors. It has been reported that astrocytes produce endothelin-1 during cerebral ischemia, which in turn stimulates endothelin ET_B receptors in microglia to produce nitric oxide. Endothelin ET_B receptor-mediated production of nitric oxide has also been observed in endothelial cells (Fuxe et al., 1989). The details of the processes from receptor stimulation to induction of nitric oxide synthase remain unclear, and this is also true for the expression of cyclooxygenase 2. These processes should be investigated in the future.

The difference in the processes leading to cyclooxygenase 2 protein expression in endothelin-1- and lipopolysaccharide-stimulated macrophages is also unclear, because combination of the two endothelin receptor antagonists had no effect on cyclooxygenase 2 protein expression and prostaglandin E_2 production in lipopolysaccharide-stimulated macrophages. It should be further studied whether the processes from stimulation to cyclooxygenase 2 protein expression in lipopolysaccharide-stimulated macrophages are similar to those in responses to endothelin-1 stimulation, because we have to consider that endothelin-1 can interact with other inflammatory stimulating factor such as lipopolysaccharide in macrophages.

Dexamethasone clearly inhibited both cyclooxygenase 2 protein expression and prostaglandin E_2 production in endothelin-1- or lipopolysaccharide-stimulated macrophages. As this agent is known to inhibit phospholipase A_2 activation and cyclooxygenase 2 expression at the protein and mRNA level (Evett et al., 1993), it is reasonable that it completely inhibited both events. Indomethacin also inhibited cyclooxygenase 2 protein expression and prostaglandin E_2 production in endothelin-1- or lipopolysaccharide-stimulated macrophages. However, the inhibitory effects on cyclooxygenase 2 protein expression were apparently less pronounced than those on prostaglandin E_2 production, and the inhibition of cyclooxygenase expression was apparently greater in endothelin-1-stimulated macrophages than in lipopolysaccharide-stimulated macrophages. It is controversial whether this agent inhibits cyclooxygenase 2 protein expression in lipopolysaccharide-stimulated macrophages (Tordjman et al., 1995; Nakatsuji et al., 1996). The present result showed that indomethacin inhibits cyclooxygenase 2 protein expression to a similar extent as reported in earlier studies (Tordjman et al., 1995). The question is why the inhibitory effect on cyclooxygenase 2 protein expression in endothelin-1-stimulated macrophages was much stronger than that seen in lipopolysaccharide-stimulated macrophages. At present, we have no answer to this question. Earlier workers also showed that the addition of prostaglandin E_2 restored the inhibitory effects of indomethacin in lipopolysaccharide-stimulated macrophages. This is understood to be a feed-forward effect. These effects of various prostanoids should be further investigated either in endothelin-1- or lipopolysaccharide-stimulated macrophages. NS398 is a cyclooxygenase 2-selective inhibitor but this agent slightly increased cyclooxygenase 2 protein expression in lipopolysaccharide-stimulated macrophages. The cause also remains to be clarified. There are no suitable agents that selectively inhibit cyclooxygenase 1. Thus, many problems remain which should be further studied pharmacologically, but the present study proved that endothelin ET_A and ET_B receptor-mediated processes occur in mouse resident peritoneal macrophages and lead to the promotion of prostaglandin E_2 production. This may be a clue to understanding the role of endothelin-1 in the pathogenesis of various diseases in which there is macrophage infiltration.

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