ELSEVIER

European Journal of Pharmacology 426 (2001) 21–24

ejp

www.elsevier.com/locate/ejphar

Short communication

Prostaglandin E_2 increases surfactant secretion via the EP_1 receptor in rat alveolar type II cells

Mohamed A.M. Morsy, Yoichiro Isohama*, Takeshi Miyata

Department of Pharmacological Sciences, Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan

Received 7 June 2001; received in revised form 6 July 2001; accepted 13 July 2001

Abstract

Prostaglandin E_2, the predominant cyclooxygenase metabolite of arachidonic acid in alveolar type II cells, can stimulate pulmonary surfactant secretion. The actions of prostaglandin E_2 are mediated by four prostaglandin E (EP) receptor subtypes designated EP_1, EP_2, EP_3 and EP_4. These subtypes couple to different signal transduction pathways. However, it is not clear which of these subtypes is expressed on type II cells and mediates surfactant secretion. We found that the four subtypes of EP receptors are expressed on the primary cultured alveolar type II cells from adult rats. We also concluded that EP_1 receptor appears to mediate prostaglandin E_2-induced surfactant secretion through Ca^{2+} mobilization. © 2001 Published by Elsevier Science B.V.

Keywords: Prostaglandin E_2 receptor; Alveolar type II cell; Pulmonary surfactant; Ca^{2+}, cytosolic free

1. Introduction

Prostaglandin E_2 exerts a variety of biological actions in various tissues and cells. These actions are mediated by specific prostaglandin E (EP) receptors, which are classified into four subtypes: EP_1, EP_2, EP_3 and EP_4. These subtypes differ in their signal transduction pathways; namely, Ca^{2+} mobilization (EP_1), and stimulation (EP_2 and EP_4) or inhibition (EP_3) of adenylate cyclase (Negishi et al., 1995).

Alveolar type II cells secrete pulmonary surfactant, a complex mixture of lipids and proteins. Its main function is to reduce the surface tension within the alveoli, and thereby lowers the work of breathing and prevents alveolar collapse at expiration (Keough, 1998). Various physiological and pharmacological agents that act via at least three different intracellular pathways regulate the secretion of pulmonary surfactant. According to the involved second messengers and protein kinases, these pathways are cyclic AMP (cAMP)/protein kinase A, diacylglycerol/protein kinase C and Ca^{2+}/Ca^{2+}-calmodulin-dependent protein kinase (Rooney et al., 1994).

Alveolar type II cells produce large amounts of lipid mediators including prostaglandin E_2, which have been suggested to stimulate pulmonary surfactant secretion (Marino and Rooney, 1980; Gilfillan and Rooney, 1985). The aim of the present study was to determine which EP receptor subtypes are expressed on alveolar type II cells and, of these, which subtypes act to mediate the surfactant secretion.

2. Materials and methods

2.1. Animals and chemicals

The rats were purchased from Kyudo (Fukuoka, Japan). Prostaglandin E_2, terbutaline sulfate and ATP disodium salt were from Sigma (St. Louis, MO, USA). ONO-8711 [6-[(2*S*,3*S*)-3-(4-chloro-2-methylphenylsulfonylaminomethyl)-bicyclo[2.2.2]octan-2-yl]-5*Z*-hexenoic acid] was a generous gift from Ono Pharmaceutical (Osaka, Japan). Fura-2 acetoxymethyl ester was from Dojin Chem. (Kumamoto, Japan). Fetal bovine serum was from JHR Bioscience (Lenexa, KS, USA).

2.2. Cell isolation and culture

Pathogen-free male Wistar rats (180–200 g) were used to isolate alveolar type II cells as described previously (Isohama et al., 1995). Briefly, lungs were cleared of blood and then digested with trypsin to obtain free cells. The

* Corresponding author. Tel.: +81-96-371-4185; fax: +81-96-362-7795.

E-mail address: isohama@gpo.kumamoto-u.ac.jp (Y. Isohama).

0014-2999/01/$ - see front matter © 2001 Published by Elsevier Science B.V.
PII: S0014-2999(01)01211-0

cells were incubated for 1 h on plates coated with rat immunoglobulin G and the unattached cells were removed and collected by centrifugation. The isolated cells were resuspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with fetal bovine serum (10% v/v), L-glutamate (2 mM), penicillin (100 units/ml) and streptomycin (100 μg/ml). The cells were then plated on 24-well tissue culture plates (1×10^6 cells/well) for measurement of phosphatidylcholine secretion and (5×10^5 cells/well) for measurement of cAMP concentration or on glass bottom culture dishes (MatTek Ashland MA, USA) (2×10^5 cells/cm^2) for measurement of cytosolic free Ca^{2+} and cultured for 22 h at 37 °C in 5% CO_2 in humidified air. [^{3}H]choline (74 kBq/ml) was added to the culture medium of cells that were to be used in surfactant secretion assay. The cell purity and viability after 22 h in primary culture were ~95% and ~98% using alkaline phosphatase staining and trypan blue dye exclusion, respectively.

2.3. Secretion of phosphatidylcholine

The cells were washed with DMEM and then equilibrated for 30 min. The used agents were added and the incubation was continued for another 90 min. The medium was then aspirated and the cells were lysed with ice-cold Triton X-100 (0.05% v/v). Lipids were extracted from both the medium and the cells according to the method of Folch et al. (1957), and the radioactivity in lipid extracts was measured by a liquid scintillation counter (Beckman LS 6500, USA). Secretion was expressed as percent of the radioactivity of [^{3}H]phosphatidylcholine in the medium over the sum of the radioactivity found in the cells plus medium.

Lactate dehydrogenase (LDH) activity was determined, as a measure of cytotoxicity, using LDH assay kit (Nippon Shoji, Osaka, Japan). The amount of LDH released into the medium did not exceed 1% of the total cell content in all experiments.

2.4. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from cultured type II cells by the guanidium thiocyanate/phenol/chloroform method. RT-PCR experiments were performed with RNA PCR kit (Takara Shuzo, Shiga, Japan) according to the manufacturer's instructions: 42 °C for 30 min, 99 °C for 5 min and 5 °C for 5 min for reverse transcription (RT). The PCR was performed using the following EP receptor-specific primers: sense, 5′ AGCGCTGCCTATCTTCTCCAT 3′ and antisense, 5′ CCAAGGCTAATGAAACACCAA 3′ for EP_1 receptor; sense, 5′ CGTGTACCTATTTCGCTTTC 3′ and antisense, 5′ GAGGTCCCACTTTTCCTTTC 3′ for EP_2 receptor; sense, 5′ TGGGTGGCGCTCACCGACTT 3′ and antisense, 5′ GCATTGCTCAACCGACATCTG 3′ for EP_3 receptor; sense, 5′ ATGTCCATCCCCGGAGTCAA 3′ and antisense, 5′ CGGACCACCACGAAGTAGCTGA 3′ for EP_4 receptor. Each PCR cycle involved denaturation at 94 °C for 1 min, annealing at 55, 45, 45 and 60 °C for 1 min for EP_1, EP_2, EP_3 and EP_4 receptors, respectively, and extension at 72 °C for 1 min except EP_4 receptor 3 min, and the reaction mixture was subjected to 35 cycles. PCR was carried out in an automated thermal cycler (Tpersonal, Biometra, UK). Amplified PCR products were electrophoresed on 1.5% agarose gels and visualized with ethidium bromide.

2.5. Measurement of cytosolic free Ca^{2+}

Cultured cells were washed and incubated in HEPES-buffer [NaCl (140 mM), KCl (5 mM), $CaCl_2$ (1 mM), $MgCl_2$ (1 mM), glucose (24 mM) and HEPES (10 mM); pH 7.4] containing fura-2 (10 μM) and bovine serum albumin (2% w/v) for 60 min. The cytosolic free Ca^{2+} of a single cell was measured using laser-excitation fluorescence microscopy system (Argus/HiSCA, Hamamatsu Photonics, Japan).

2.6. Measurement of cAMP

The cells were washed with DMEM and preincubated for 30 min. The used agents were then added and the incubation continued for 5 min. The medium was aspirated and cellular cAMP was quickly extracted with ice-cold HCl (0.1 N). The HCl extract was immediately frozen, lyophilized and stored at −70 °C. The cAMP contents were determined using cAMP enzyme immunoassay kit (Biotrak, Amersham Pharmacia Biotech, UK) following the manufacturer's protocol.

2.7. Statistics

All data are means ± S.D. Difference between groups were tested by Duncan New Multiple Range test. Significance was accepted at $P < 0.05$.

3. Results

RT-PCR was used to detect the expression of EP receptor subtypes in cultured rat alveolar type II cells. As shown in Fig. 1A, signals indicating the presence of mRNAs encoding EP_1, EP_2, EP_3 and EP_4 receptors were detected by identity with the predicted product sizes of 419, 568, 669 and 522 bp, respectively. RT-negative reactions yielded no products.

Second messenger signal transduction via EP receptors was assessed by measuring both changes in cAMP production and cytosolic free Ca^{2+} level in alveolar type II cells. Prostaglandin E_2 (10^{-7} M) had no significant effect on the cellular cAMP content (Fig. 1B) despite the apparent

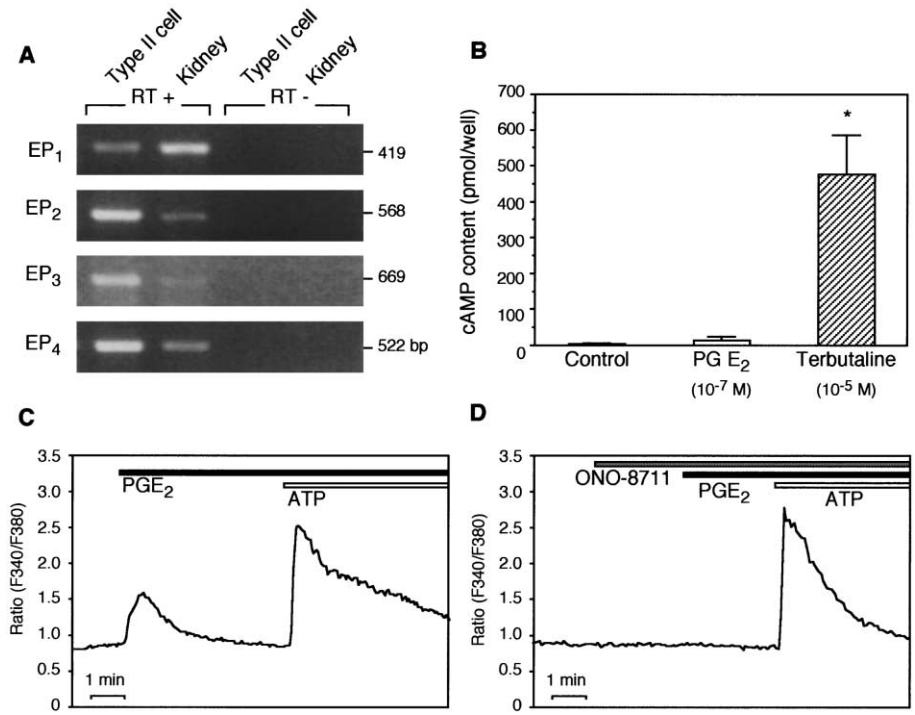

Fig. 1. Expressions and functions of EP_1, EP_2, EP_3 and EP_4 receptors in cultured rat alveolar type II cells. (A) RNA samples were reverse-transcribed to cDNA and amplified using specific primers for EP_1, EP_2, EP_3 and EP_4 receptors. Total RNA from kidney was used as a positive control. All PCR products were found to be in the predicted size. The reactions were carried out in the presence (+) or absence (−) of reverse transcriptase. (B) Effect of prostaglandin E_2 (10^{-7} M) on cAMP production. Cultured cells were washed and preincubated for 30 min in fresh medium. Prostaglandin E_2 and terbutaline (10^{-5} M) (a positive control) were then added and the incubation continued for 5 min, after which the cAMP content of the cells was measured. Values are means ± S.D. from three experiments. $^{*}P < 0.05$ vs. control. (C) Effect of prostaglandin E_2 (10^{-7} M) on cytosolic free Ca^{2+} level. Cells were loaded with fura-2 for 60 min. ATP was used as a positive control. (D) Effect of ONO-8711, a selective EP_1 receptor antagonist (10^{-7} M) on cytosolic free Ca^{2+} level. PGE_2 = Prostaglandin E_2.

response of cells to terbutaline (10^{-5} M) (a positive control). On the other hand, Fig. 1C shows the time course of intracellular Ca^{2+} release after treatment with prostaglandin E_2 (10^{-7} M) and ATP (10^{-3} M) (as a positive control), respectively. Prostaglandin E_2 markedly increased cytosolic free Ca^{2+} compared to the basal level. We also examined the effect of a selective EP_1 receptor antagonist (ONO-8711) on cytosolic free Ca^{2+} level. ONO-8711 (10^{-7} M) completely prevented cytosolic free Ca^{2+} increase by prostaglandin E_2 (10^{-7} M) (Fig. 1D).

Table 1
Effect of prostaglandin E_2 (10^{-10}–10^{-7} M) on phosphatidylcholine secretion in cultured rat alveolar type II cells
After 22 h in culture, the cells were incubated for 90 min with the indicated concentrations. Percent phosphatidylcholine secretion rate was measured as described under Materials and methods. In the control incubation, 0.43 ± 0.13% (mean ± S.D.) of [^{3}H]phosphatidylcholine was released into the medium in 90-min incubation. Each result was represented as % of control.

Treatment	Secretion (% of control)
Control	100
Prostaglandin E_2 (10^{-10} M)	126.89 ± 62.41
Prostaglandin E_2 (10^{-9} M)	148.38 ± 45.90
Prostaglandin E_2 (10^{-8} M)	188.11 ± 35.79[a]
Prostaglandin E_2 (10^{-7} M)	196.90 ± 20.16[a]
ONO-8711 (10^{-7} M)	113.24 ± 65.81
Prostaglandin E_2 (10^{-7} M) + ONO-8711 (10^{-7} M)	96.71 ± 16.50[b]
Terbutaline (10^{-3} M)	211.87 ± 36.75[a]

[a] Values are mean ± S.D. from 3–4 experiments.
[a and b] $P < 0.05$ vs. control and prostaglandin E_2 (10^{-7} M), respectively.

The effect of increasing concentrations (10^{-10}–10^{-7} M) of prostaglandin E_2 on surfactant secretion is shown in Table 1. Exogenously added prostaglandin E_2 had significantly stimulated surfactant secretion in a concentration-dependent manner. The role of EP_1 receptor in surfactant secretion was further defined by using the EP_1 receptor antagonist, ONO-8711. The EP_1 receptor antagonist (10^{-7} M) completely inhibited surfactant secretion induced by prostaglandin E_2 (10^{-7} M) (Table 1).

4. Discussion

Prostaglandin E_2 is one of the major components of arachidonic acid metabolism in alveolar type II cells. It is synthesized both constitutively and inducibly. In addition, prostaglandin E_2 is released from other lung cells that

interact with alveolar type II cells in a paracrine fashion (Panos et al., 1992). Several studies have suggested a role for prostaglandins including prostaglandin E_2 in the regulation of pulmonary surfactant secretion. Marino and Rooney (1980) examined surfactant secretion in newborn rabbit lung slices. They found that the basal rate of surfactant secretion was inhibited by indomethacin and flufenamic acid and stimulated by prostaglandin E_2. In addition, arachidonic acid had stimulated phosphatidylcholine secretion in primary cultures of adult rat type II cells. The stimulatory effect is diminished by lipoxygenase and cyclooxygenase inhibitors (Gilfillan and Rooney, 1985). We found that direct application of prostaglandin E_2 (10^{-7} M) doubled the surfactant secretion in purified alveolar type II cells. To our knowledge, this is the first direct evidence for the regulatory effect of prostaglandin E_2 on surfactant secretion.

Our results suggest that the stimulatory effect of prostaglandin E_2 on surfactant secretion is EP_1 receptor-dependent. This is supported by the following findings: (1) prostaglandin E_2 increased cytosolic free Ca^{2+} that is coupled to EP_1 receptor activation and at the same time it is an important second messenger in surfactant secretion; (2) ONO-8711, a selective EP_1 receptor antagonist, completely inhibited the increase in both phosphatidylcholine secretion and cytosolic free Ca^{2+} induced by prostaglandin E_2; (3) in agreement with a previous report (Skinner et al., 1989), prostaglandin E_2 did not affect cAMP content although EP_2, EP_3, and EP_4 receptors are known to couple to this second messenger. Rose et al. (1999) reported that prostacyclin enhanced stretch-induced surfactant secretion in alveolar type II cells and this enhancement was concomitant with increase in cAMP content. Therefore, we assume that underlying mechanisms for the prostacyclin-induced enhancement are through different receptor(s) other than EP_1 receptor.

Prostaglandin E_2 is characterized by the wide distribution of its receptors in the body (Negishi et al., 1995); however, little is known about the expression of these receptors in alveolar type II cells. Our data revealed that all four EP receptor subtypes are expressed on cultured rat alveolar type II cells. The role of EP_2, EP_3 and EP_4 receptors in adult alveolar type II cells is still not clear. However, in human fetal lung explants prostaglandin E_2 has been reported to induce surfactant protein A gene expression through an increase in cAMP formation (Acarregui et al., 1990). This increase is characteristically coupled to both EP_2 and EP_4 receptors. In addition, Mukhopadhyay and Dutta-Roy (1998) reported that EP_3 receptor is expressed in guinea pig fetal type II cells apical membrane. They suggested that prostaglandin E_2 stimulates alveolar Na^+ transport via this receptor. Taking together the present and the previous reports, it seems possible that different EP receptor subtypes have different functions in alveolar type II cells and that EP_1 receptor-induced surfactant secretion appears to be the predominant function of prostaglandin E_2 in these cells.

References

Acarregui, M.J., Snyder, J.M., Mitchell, M.D., Mendelson, C.R., 1990. Prostaglandins regulate surfactant protein A (SP-A) gene expression in human fetal lung in vitro. Endocrinology 127, 1105–1113.

Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Gilfillan, A.M., Rooney, S.A., 1985. Arachidonic acid metabolites stimulate phosphatidylcholine secretion in primary cultures of type II pneumocytes. Biochim. Biophys. Acta 833, 336–341.

Isohama, Y., Matsuo, T., Kai, H., Takahama, K., Miyata, T., 1995. Changes in β_1- and β_2-adrenoceptor mRNA levels in alveolar type II cells during cultivation. Biochim. Mol. Biol. Int. 36, 561–568.

Keough, K.M.W., 1998. Surfactant composition and extracellular transformations. In: Rooney, S.A. (Ed.), Lung Surfactant: Cellular and Molecular Processing. R.G. Landes, Austin, TX, pp. 1–27.

Marino, P.A., Rooney, S.A., 1980. Surfactant secretion in a newborn rabbit lung slice model. Biochim. Biophys. Acta 620, 509–519.

Mukhopadhyay, S., Dutta-Roy, A., 1998. G protein-coupled prostaglandin receptor modulates conductive Na+ uptake in lung apical membrane vesicles. Am. J. Physiol. 274, L567–L572.

Negishi, M., Sugimoto, Y., Ichikawa, A., 1995. Prostaglandin E receptors. J. Lipid Mediators Cell Signalling 12, 379–391.

Panos, R.J., Voelkel, N.F., Cott, G.R., Mason, R.J., Westcott, J.Y., 1992. Alterations in ecosanoid production by rat alveolar type II cells isolated after silica-induced lung injury. Am. J. Respir. Cell Mol. Biol. 6, 430–438.

Rooney, S.A., Young, S.L., Mendelson, C.R., 1994. Molecular and cellular processing of lung surfactant. FASEB J. 8, 957–967.

Rose, F., Zwick, K., Ghofrani, H.A., Sibelius, U., Seeger, W., Walmrath, D., Grimminger, F., 1999. Prostacyclin enhances stretch-induced surfactant secretion in alveolar epithelial type II cells. Am. J. Respir. Crit. Care Med. 160, 846–851.

Skinner, S.J.M., Lowe, C., Ashby, C.J., Liggins, G.C., 1989. Effects of corticosteroids, prostaglandin E_2, and beta-agonists on adenylate cyclase activity in fetal rat lung fibroblasts and type II epithelial cells. Exp. Lung Res. 15, 335–343.