The effect of prostaglandin E_2 on cystine uptake and glutathione synthesis by human lung fibroblasts

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Abstract

Prostaglandin E_2 (PGE$_2$) is an inflammatory mediator capable of regulating fibroblast cell proliferation, matrix protein production, and system A amino acid transport. System x_c^- amino acid transport is regulated by electrophilic agents and oxygen. The effect of PGE$_2$ on the x_c^- system transport of cystine and the synthesis of glutathione by human lung fibroblasts was examined. Preincubation of fibroblast cultures with PGE$_2$ decreased cystine uptake by 42%. Kinetic studies revealed a 42% decrease in the V_{max} of the x_c^- system transporter in PGE$_2$-treated fibroblasts; however, the apparent K_m was not affected. The glutathione content of PGE$_2$-treated fibroblasts was decreased by up to 25% of control. These results demonstrate that system x_c^- transport of cystine is regulated by PGE$_2$ and suggest that the limited availability of intracellular cysteine inhibited glutathione synthesis. © 1998 Published by Elsevier Science B.V. All rights reserved.

Keywords: Prostaglandin E_2; Cystine; Glutathione; Human lung fibroblast

1. Introduction

The transport of amino acids across the mammalian cell membrane is mediated by specific amino acid transport systems [1]. Transport systems exhibit broad and overlapping substrate specificity. Most cell types express the Na$^+$-independent transport system L and the Na$^+$-dependent transport systems A and ASC. System L transports branch-chain amino acids whereas systems A and ASC transport aliphatic amino acids. Different cell types may express additional transport systems. Fibroblasts express system ASC for the transport of alanine, serine, and cysteine [2]. Fibroblasts also express system x_c^-, a Na$^+$-independent system highly specific for cystine and glutamate [3–5]. A variety of substances and cell culture conditions can regulate amino acid transport systems [1].

Prostaglandin E_2 (PGE$_2$) was shown previously to regulate system A amino acid transport by intact cells [6]. In lung fibroblasts, PGE$_2$ decreased the transport of proline and aminoisobutyric acid by system A; however, the transport of leucine by system L was not affected [6]. In hepatic plasma membrane vesicles, PGE$_2$ increased the transport of alanine by system A and glutamine by system N [7] and the Na$^+$-independent transport of arginine by system y$^+$ [8].

Human lung fibroblasts transport cystine almost exclusively by the x_c^- system in which the influx of

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cystine is coupled to the efflux of glutamate [3–5]. Following uptake, cystine is rapidly reduced to cysteine in the intracellular space [9,10]. The disulfide form, cystine, is predominant outside the cell whereas the sulfhydryl form, cysteine, is predominant inside the cell. Cysteine is present at very low concentration in the extracellular space and may be transported directly by the ASC system.

Intracellular cysteine is necessary for the synthesis of proteins, glutathione, and a variety of bioactive metabolites. Glutathione synthesis is limited by the availability of intracellular cysteine and is regulated by changes in cystine transport activity [2,11–13]. Cystine transport by system x_c^- is induced by fibroblasts incubated in electrophilic agents [14] and by endothelial cells [15], macrophages [16], and fibroblasts [17] exposed to hyperoxia. Cystine also regulates the synthesis of $\alpha 1(I)$ collagen mRNA by fibroblasts [18]. In humans, low plasma cystine levels are associated with human immunodeficiency virus infection, cancer, and senescence [19].

In these studies, the effect of PGE_2 on cystine uptake and glutathione synthesis by human lung fibroblasts was examined. Preincubation of fibroblast cultures with PGE_2 decreased cystine uptake and glutathione synthesis; however, cysteine uptake was not affected.

2. Materials and methods

2.1. Cells and tissue cultures

Human embryonic lung fibroblasts (IMR-90, Institute for Medical Research, Camden, NJ) were grown in Dulbecco's modified Eagle's medium (DMEM) with 0.37 g sodium bicarbonate/100 ml, 10% fetal bovine serum, 100 U penicillin/ml, 10 μg streptomycin/ml, 1 mM sodium pyruvate, and 0.1 mM nonessential amino acids. After confluence was reached, the serum content of the medium was reduced to 0.4%. Cell numbers were determined in triplicate with an electronic particle counter (Coulter Counter ZM).

2.2. Cystine transport

Fibroblasts were incubated in fresh medium defi-cient in all amino acids except labeled cystine for the indicated time periods at 37°C [6]. The incubation was terminated by rinsing the cells three times with cold Puck's saline. Following the incubation, the cellular material was extracted with 1 ml of 10% trichloroacetic acid. The amount of trichloroacetic acid-soluble labeled cystine was determined by scintillation counting. The trichloroacetic acid-insoluble fraction was used for protein determinations [6].

2.3. Glutathione assay

Fibroblasts were trypsinized, counted and resuspended. Proteins were precipitated by the addition of 0.5% perchloric acid, sonication and centrifugation at $3000 \times g$ for 20 min at 4°C. The supernatants were adjusted to pH 7 with 1 M KOH/0.3 M MOPS and centrifuged at $10\,000 \times g$ for 5 min at 4°C. The glutathione content was determined by a colorimetric assay using the method of Tietze [20] with modifications described by Akerboom and Sies [21]. Each assay was calibrated with standard glutathione. Glutathione content was expressed as nmol per 10^6 cells.

2.4. Statistics

A Student's t-test was used for means of unequal size [22]. Probability values less than 0.05 were considered significant.

3. Results

The effect of PGE_2 on cystine uptake by human lung fibroblasts was examined using fibroblast cultures incubated in medium containing PGE_2 (0.1 μM) for 24 h. In quiescent lung fibroblast cultures, the uptake of [^{35}S]cystine remained linear for at least 10 min (Fig. 1). Preincubation of the fibroblast cultures with PGE_2 decreased [^{35}S]cystine uptake by 42% ($P < 0.01$) at 10 min.

The effect of PGE_2 on cysteine uptake by human lung fibroblasts was examined in similar experiments. As noted previously, cysteine is transported primarily by system ASC. In quiescent lung fibroblast cultures, the uptake of [^{35}S]cysteine also remained linear for at least 10 min (Fig. 2). Preincubation of the fibroblast
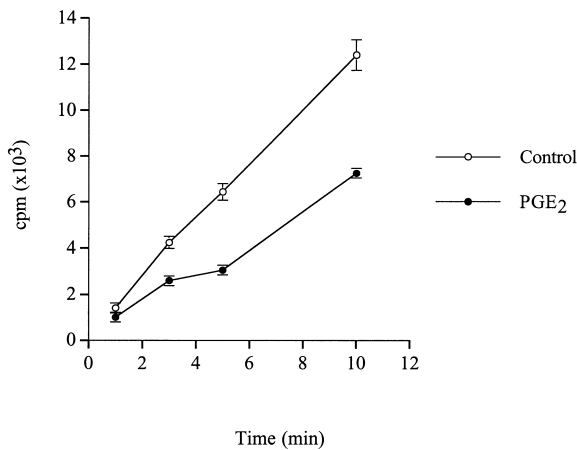
Fig. 1. Effect of PGE$_2$ on cystine uptake by lung fibroblasts. Quiescent lung fibroblast cultures were incubated in DMEM in the presence (●) or absence (○) of PGE$_2$ (0.1 µM) for 24 h. The medium was replaced with amino acid deficient medium containing [^{35}S]cystine (1 µCi/mM) for the indicated time periods. The uptake of [^{35}S]cystine was determined following the extraction of cellular material with 10% trichloroacetic acid. Data are expressed as cpm, mean ± 1 S.E.; $n = 3$.

Fig. 2. Effect of PGE$_2$ on cysteine uptake by lung fibroblasts. Quiescent lung fibroblast cultures were incubated in DMEM in the presence (●) or absence (○) of PGE$_2$ (0.1 µM) for 24 h. The medium was replaced with amino acid deficient medium containing [^{35}S]cysteine (1 µCi/mM) for the indicated time periods. The uptake of [^{35}S]cysteine was determined following the extraction of cellular material with 10% trichloroacetic acid. Data are expressed as cpm; $n = 2$.

cultures with PGE$_2$, however, did not affect [^{35}S]cysteine uptake.

To assess the dose–response relation of PGE$_2$ on cystine uptake by human lung fibroblasts, fibroblast cultures were incubated in medium containing PGE$_2$ at various concentrations for 24 h and the uptake of [^{35}S]cystine was determined. Preincubation of the fibroblast cultures with increasing concentrations of PGE$_2$ resulted in dose-dependent decreases in [^{35}S]cystine uptake (Fig. 3). The uptake at 0.01 µM, 0.1 µM, and 1 µM was decreased by 12%, 46%, and 68% of control, respectively.

The effect of PGE$_2$ on cystine uptake by human lung fibroblasts was examined further using kinetic studies. Fibroblast cultures were incubated in medium containing PGE$_2$ (0.1 µM) for 24 h and the uptake of [^{35}S]cystine at various concentrations was determined after 10 min. Preincubation of the fibroblast cultures with PGE$_2$ resulted in a 42% decrease in the V_{max}, but had no effect on the apparent K_m (Fig. 4). The V_{max} in control and PGE$_2$-treated cultures were 1.27 and 0.74 nmol/min per mg protein, respectively. The apparent K_m in control and PGE$_2$-treated cultures were 0.053 and 0.056 mM, respectively.

The relation of cystine on glutathione synthesis by human lung fibroblasts was assessed using fibroblast
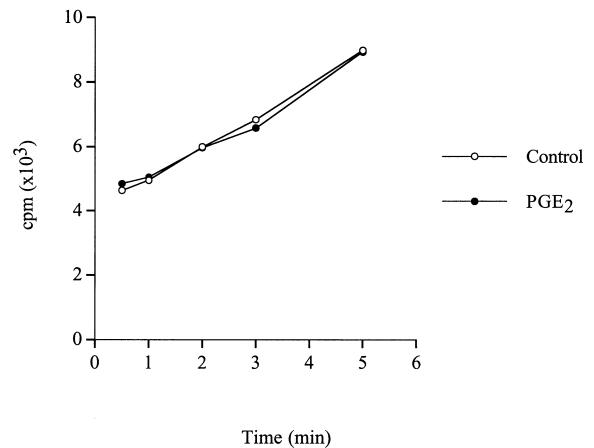
cultures incubated in medium containing cystine at various concentrations for 24 h. Fibroblast cultures incubated in increasing concentrations of cystine (0 to 2 mM) resulted in concentration-dependent increases in the glutathione content (Fig. 5). The glutathione content of fibroblast cultures incubated in DMEM
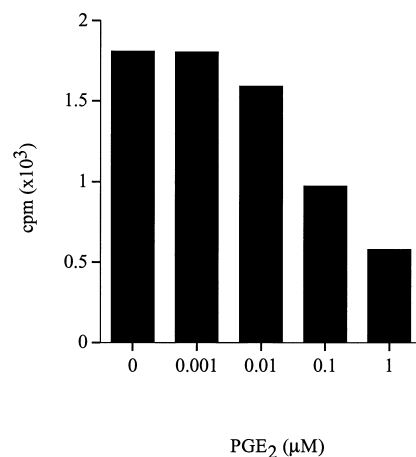

Fig. 3. Dose–response relation of PGE$_2$ on cystine uptake by lung fibroblasts. Quiescent lung fibroblast cultures were incubated in DMEM in the presence of PGE$_2$ at the indicated concentrations for 24 h. The medium was replaced with amino acid deficient medium containing [^{35}S]cystine (1 µCi/mM) for 10 min. The uptake of [^{35}S]cystine was determined following the extraction of cellular material with 10% trichloroacetic acid. Data are expressed as cpm; $n = 2$.
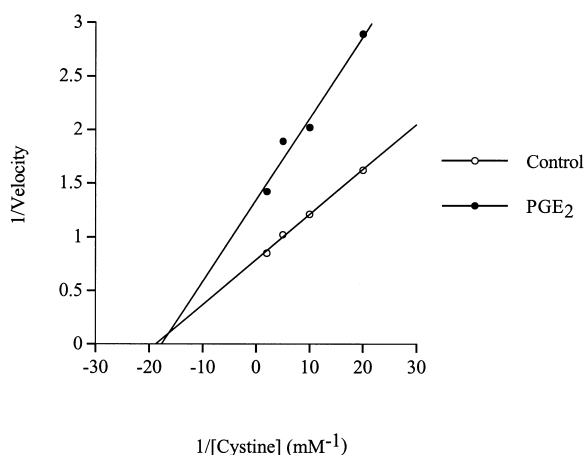
Fig. 4. Reciprocal velocity of cystine uptake plotted against reciprocal of cystine concentration (Lineweaver–Burk plot). Quiescent lung fibroblast cultures were incubated in DMEM in the presence (●) or absence (○) of PGE_2 (0.1 μM) for 24 h. The uptake of $[^{35}S]$cystine was determined following the addition of amino acid deficient medium containing $[^{35}S]$cystine at the indicated concentrations for 10 min. Velocity is expressed as nmol/min per mg protein; $n = 2$. The V_{max} in control and PGE_2-treated cultures were 1.27 and 0.74 nmol/min per mg protein, respectively. The apparent K_m in control and PGE_2-treated cultures were 0.053 and 0.056 mM, respectively.

(0.2 mM cystine) was 6.51 ± 0.24 nmol/10^6 cells (mean ± 1 S.D.; $n = 3$).

Fibroblast cultures were then incubated in medium containing PGE_2 at various concentrations for 24 h and the glutathione content was determined. Prein-
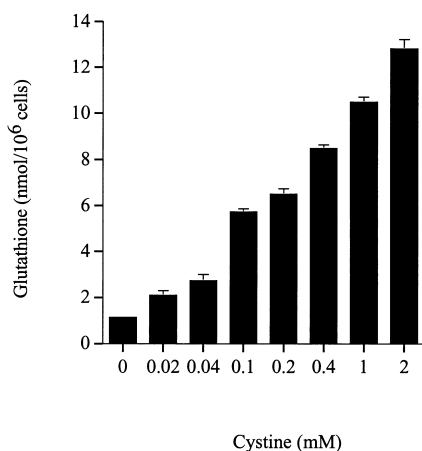

Fig. 5. Dose–response relation of cystine on glutathione synthesis by lung fibroblasts. Quiescent lung fibroblast cultures were incubated in DMEM containing cystine at the indicated concentrations for 24 h. The glutathione content was then determined by a colorimetric assay. Data are expressed as nmol/10^6 cells, mean $\pm$ S.D.; n=3.
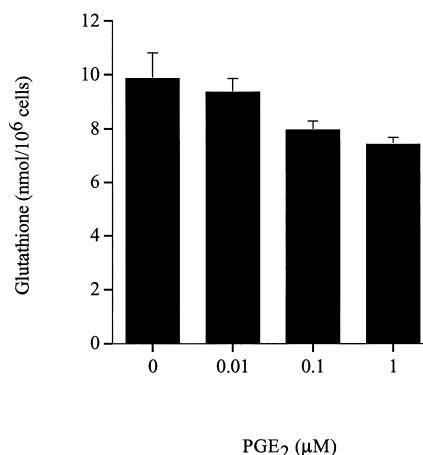

Fig. 6. Effect of PGE_2 on glutathione synthesis by lung fibroblasts. Quiescent lung fibroblast cultures were incubated in DMEM in the presence of PGE_2 at the indicated concentrations for 24 h. The glutathione content was then determined by a colorimetric assay. Data are expressed as nmol/10^6 cells, mean $\pm$ S.D.; $n = 3$.

cubation of the fibroblast cultures with increasing concentrations of PGE_2 resulted in progressive decreases in the glutathione content (Fig. 6). The glutathione content at 0.1 μM and 1 μM was decreased by 19% ($P < 0.05$) and 25% ($P < 0.05$) of control, respectively.

4. Discussion

The addition of PGE_2 to human lung fibroblast cultures decreased cystine uptake by the x_c^- system; however, cysteine uptake by the ASC system was not affected. The uptake of cystine is dependent on the activity of the x_c^- system in which extracellular cystine is transported in exchange for intracellular glutamate [3–5]. System x_c^- transport of cystine is induced by electrophilic agents and hyperoxia [14–18]; however, this is the first demonstration that it is regulated by an inflammatory mediator.

Cell membrane proteins involved in the transport of amino acids have the same kinetic behavior as enzymes. Kinetic studies of the x_c^- system revealed a decrease in the V_{max} in PGE_2-treated fibroblasts, reflecting a decrease in the number of functional x_c^- transporters. The observed effect may have been due to the degradation of transport proteins or to the activation of transport protein inhibitors. There

was no change in the apparent K_m of the x_c^- system in PGE$_2$-treated fibroblasts, indicating there was no change in the affinity of the x_c^- transporter for its substrate cystine. The V_{max} and the apparent K_m of the x_c^- system in untreated fibroblasts were in agreement with previous reports [14]. Interestingly, PGE$_2$ inhibits system A transport of amino acids by fibroblasts primarily by increases in the apparent K_m and not by changes in the V_{max} [6].

The synthesis of glutathione was shown to be dependent on the extracellular concentration of cystine. Following uptake, cystine is rapidly reduced to cysteine which then becomes available for glutathione synthesis. Glutathione synthesis is regulated by alterations in the activity of the x_c^- system transport of cystine [11–13]. The current data suggest that fibroblasts treated with PGE$_2$ decreased glutathione synthesis as a result of system x_c^- inhibition and the subsequent decrease in intracellular cysteine availability. In phytohemagglutinin-stimulated human mononuclear cells, PGE$_2$ decreased glutathione synthesis; however, the mechanism was not elucidated [23]. The decreased uptake of cystine may have accounted for the observed effect.

The synthesis of PGE$_2$ from arachidonic acid involves reactions catalyzed by prostaglandin H synthase and subsequently by glutathione-dependent PGE$_2$ isomerases [24–28]. Glutathione depletion inhibits PGE$_2$ synthesis by fibroblasts [29]. Oxidant stress also decreases PGE$_2$ synthesis and this effect is enhanced by depleting intracellular glutathione [29]. It is possible that PGE$_2$-induced depletion of glutathione may limit further PGE$_2$ synthesis. The role of glutathione as a mediator in the feedback inhibition of PGE$_2$ is currently under investigation. In summary, these studies demonstrate that PGE$_2$ inhibited system x_c^- transport of cystine by human lung fibroblasts and suggest that the limited availability of intracellular cysteine inhibited glutathione synthesis.

Acknowledgements

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