ISSN: 0976-3104
SPECIAL ISSUE: REDOX BIOLOGY IN CARDIOVASCULAR AND NEUROLOGICAL DISORDERS

ROLE OF PROTEIN KINASE C-α IN LEUKOTRIENE D_4 - MEDIATED STIMULATION OF CYTOSOLIC PHOSPHOLIPASE A_2 IN PULMONARY SMOOTH MUSCLE CELLS

Sajal Chakraborti*, Tapati Chakraborti, Md. Nur Alam, Soni Shaikh, and Soumitra Roy
Department of Biochemistry and Biophysics, University of Kalyani, Kalyani–741235, West Bengal, INDIA

Received on: 23-Mar-2011; **Revised on:** 29th-Mar-2011; **Accepted on:** 02th-April-2011; **Published on:** 16th-Aug-2010.
***Corresponding author:** Email: saj_chakra@rediffmail.com Tel: +91-9831228224; Fax: +91-033-25828282

ABSTRACT

We investigated the mechanism by which LTD_4 stimulates PLA_2 activity and the role of protein kinase C (PKC) in this scenario. Treatment of bovine pulmonary artery smooth muscle cells with LTD_4 stimulated an aprotinin-sensitive protease activity, PKC activity, and PLA_2 activity in the cell membrane. Pretreatment with vitamin E, dithiothreitol, aprotinin (serine protease inhibitor), BAPTA-AM (intracellular Ca^{2+} chelator), Go6976 (PKC-α inhibitor) and $AACOCF_3$ ($cPLA_2$ inhibitor) prevented LTD_4 stimulated PLA_2 activity. Immunoblot studies of the cell membrane isolated from LTD_4 stimulated cells with $cPLA_2$ antibody elicited a marked increase in the immunoreactive protein profile. Immunoblot study with PKC-α antibody showed an additional 47-kDa immunoreactive band and that was prevented upon pretreatment of the cells with aprotinin. These results suggest that LTD_4 caused an increase in reactive oxidants species (ROS), which subsequently stimulated an aprotinin sensitive protease activity and that proteolytically activated PKC-α and consequently stimulated $cPLA_2$ activity in the cell membrane.

Keywords: *Leukotriene D_4; cytosolic phospholipase A_2; aprotinin; protein kinase-Cα; pulmonary artery smooth muscle cells*

[I] INTRODUCTION

Activation of phospholipase A_2 (PLA_2) with subsequent release of arachidonic acid (AA) is an important physiological and pathological event. Several PLA_2s were identified and are classified mainly into three groups: (i) cytosolic PLA2 ($cPLA_2$); (ii) secretory PLA_2 ($sPLA_2$); and (iii) intracellular PLA_2 ($_iPLA_2$). Cellular injury may cause a rise in intracellular Ca^{2+} level, activation of protein kinase C (PKC), and subsequently stimulation of PLA_2 activity, resulting in release of AA and its metabolites, for example, leukotrienes (LTs), which cause further injury to cells and tissues [1]. Leukotrienes especially LTD_4 have been shown to cause pulmonary hypertension and an increase in vascular permeability in isolated rabbit lungs [2]. LTs have been shown to produce oxidants, for example, superoxide radicals and activates NADPH oxidase in some systems [3, 4]. LTs have also been shown to increase $[Ca^{2+}]_i$ in different cells [5]. Previous research indicated that oxidant-mediated pulmonary hypertension occurs with the involvement of an increase in $[Ca^{2+}]_i$ [6]. Intracellular Ca^{2+} chelators, for example, TMB-8 {8-(diethylamino) octyl 3,4,5-trimethoxybenzoate} has been shown to prevent oxidant–mediated pulmonary hypertension in isolated lungs [6]. LTD_4 has also been shown to stimulate PLA_2 activity in pulmonary artery endothelial cells [7]. However, the mechanism by which LTD_4 activates PLA_2 in pulmonary artery smooth muscle cells is currently unknown.

Activation of PKC has been shown to be involved in signal regulation of many physiological and pathological processes [8]. PKC has multiple isoforms, which are cell and tissue specific [9]. PKC exists as a family of at least 12 distinct isoforms. The conventional PKC isoforms (cPKC: α, β and γ) require Ca^{2+} metabolites. The novel PKC isoforms (nPKC: δ, ε, η, and θ) require P-lipid or its metabolites; while the atypical PKC isoforms (aPKC: ζ , λ, and ι) require neither Ca^{2+} nor P-lipid or its metabolites and Ca^{2+} [10].

Proteolytic processes play important roles in experimentally induced or physiologically occurring changes in cells and tissues [11]. Aprotinin, a serine protease inhibitor, has been shown to prevent pulmonary hypertension and edema caused by a variety of stimulants [11]. Previous reports have also indicated that endogenous proteases, for example, trypsin-like proteases proteolytically activate PKC [12]. In view of this and to gain an insight into the biochemical mechanisms

www.iioab.org
THE IIOAB JOURNAL
www.iioab.webs.com
EDITED BY – PROF. S.K. MAULIK; MD, PHD
RESEARCH

THE IIOAB JOURNAL ISSN: 0976-3104

www.iioab.org

THE IIOAB JOURNAL

www.iioab.webs.com

associated with the activation of $cPLA_2$ under LTD_4 triggered condition in bovine pulmonary artery smooth muscle cells, we tested the hypothesis that LTD_4 -mediated stimulation of an aprotinin-sensitive protease plays a crucial role in activating PKC-α and subsequently stimulating $cPLA_2$ activity in the cell membrane.

[II] MATERIALS AND METHODS

Cell culture supplies and other chemicals and reagents were obtained from Sigma Chemical Co., St. Louis, MO. [14_C]AA, L-3-phosphatidyl choline-1-stearoyl-2-[1-14_C] arachidonyl and [γ-^{32}P]ATP were the products of New England Nuclear, Wilmington, DE. Antigens and polyclonal antibodies of cPLA2, PKC-α, PKC-β and PKC-γ were the products of Chemicon International (Temelcula, CA), ABCAM (Cambridge, UK), and Invitrogen Life Technologies (Carlsbad, CA).

2.1. Cell culture

Bovine pulmonary artery smooth muscle cells obtained from Cell Sciences (San Diego, CA) were studied between passages 6 and 12. All experiments were performed in serum-free media.

2.2. Preparation of cell membrane fraction

The smooth muscle cell membranes were isolated by following the procedure previously described [13].

2.3. Measurement of Ca^{2+} mobilization

$[Ca^{2}+]_i$ in the cells was determined using the fluorescent probe fura-2 [14]. Cells were incubated in serum-free DMEM, and fura-2AM was added to give a final concentration of 5 μM, kept for 2 min, and then washed to free of excess probe. Then LTD_4 (10nM) was added for 10 min and fluorescence were determined at cell concentration of 10^5 cells per milliliter (λex = 337 nm, λem = 510 nm).

2.4. Assay of protease activity

Protease activity was assessed by determining the hydrolysis of the synthetic substrate BAPNA as previously described [14]. To measure LTD_4 mediated increase in the protease activity, cells were exposed to LTD_4 (10nM) for 10 min. The membrane fraction was isolated and protease activity was measured. Vitamin E (1 mM), dithiothreitol (1mM), aprotinin (10 μg/mL), calphostin C (1 μM), Go6976 (1 μM), $AACOCF_3$ (10 μM), Bel (10 μM), and BAPTA-AM (50 μM) were added to the cells for 20 min followed by treatment with LTD_4 (10nM) for 10 min. The cell membrane fraction was isolated, and the protease activity was determined.

2.5. Assay of PLA_2 activity

PLA_2 activity was assayed using L-3-phosphatidyl choline- 1-stearoyl-2-[1-14_C] arachidonyl as the substrate [1]. The cells were treated with LTD_4 (10nM) for 10 min, then the cell membrane fraction was isolated and PLA_2 activity was measured. Vitamin E (1 mM), DTT (1mM), aprotinin (10 μg/ml), calphostin C (1 μM), Go6976 (1 μM), $AACOCF_3$ (10 μM), bromoenol lactone (Bel) (10 μM) and BAPTA-AM (50 μM) were added for 20 min followed by treatment with LTD_4 (10nM) for 10 min. The membrane fractions were isolated and PLA_2 activity was determined.

2.6. Immunoblot assay for the determination of $cPLA_2$

Cytosolic PLA_2 was detected in the membrane fraction isolated from the smooth muscle cells using polyclonal antibody of $cPLA_2$ by Western immunoblot assay [15]. Cells were treated with LTD_4 (10nM) for 10 min, then the membrane fraction was immunoblotted with the polyclonal $cPLA_2$ antibody. The cells were pretreated with aprotinin (10 μg/ml), calphostin C (1 μM), Go6976 (1 μM), $AACOCF_3$ (10 μM) and BAPTA-AM (50 μM) for 20 min followed by treatment with LTD_4, then the membrane fractions were isolated and immunoblotted with $cPLA_2$ antibody.

2.7. Measurement of PKC activity

PKC activity in the cell membrane fraction was determined by following the procedure of Kitano et al. [16]. To determine the effect of LTD_4 on membrane PKC activity, the smooth muscle cells were treated with LTD_4 (10nM) for 10 min. The membrane fraction was isolated, then PKC activity was determined. Aprotinin (10μg/ml), calphostin C (1μM), Go6976 (1μM), $AACOCF_3$ (10 μM), Bel (10 μM), and BAPTA-AM (50 μM) were added to the cells for 20 min followed by addition of LTD_4 (10nM) for 10 min. The membrane fraction was isolated and PKC activity was determined.

2.8. Immunoblot assay of PKC subspecies in the cell membrane fractions

PKC subspecies in the membrane fraction were assayed using polyclonal α, β, and γ PKC antipeptide antibodies by Western immunoblot method.

2.9. Estimation of proteins

Proteins were estimated by BCA protein assay reagent using bovine serum albumin as the standard [17].

2.10. Cell viability

The dose and time of incubation of the agents did not affect the cell viability as assessed by trypan blue exclusion.

2.11. Statistical analysis

Data were analyzed by unpaired t test and analysis of variance followed by the test of least significant difference [18] for comparisons within and between the groups, and $p < 0.05$ was considered as significant.

[III] RESULTS

The smooth muscle cell membrane fraction was characterized by following our previously described procedure [13] (data not shown). We have previously demonstrated the presence of aprotinin in pulmonary artery smooth muscle [13].

Pretreatment of the cells with vitamin E, dithiothreitol (DTT) and aprotinin prevent LTD_4 induced increase in the protease activity, PKC activity and PLA_2 activity in the cell membrane **[Table–1]**. Calphostin C (a general PKC inhibitor) inhibited PKC activity and PLA_2 activity caused by LTD_4 **[Table–2]**, without producing any significant change in the protease activity in the cell membrane **[Table–2]**. Pretreatment of the

EDITED BY – PROF. S.K. MAULIK; MD, PHD

THE IIOAB JOURNAL ISSN: 0976-3104

cells with the PKC-α inhibitor, Go6976 inhibited PKC activity and PLA_2 activity without causing any significant change in the protease activity **[Table–2]**.

Results in the parentheses indicate percent change over basal value. Protease activity is expressed as the change in absorbance at 410 nm/mg protein/30 min. PKC activity is expressed as pmol/mg protein/min; $cPLA_2$ activity is expressed as pmol AA/mg protein/min. [a]p<0.001 compared with basal condition; [b]p<0.01 compared with basal condition; [c]p<0.001 compared with LTD_4 treatment.

The $cPLA_2$ inhibitor $AACOCF_3$, but not the $iPLA_2$ inhibitor Bel, reduced basal and LTD_4 induced increase in the PLA_2 activity without causing any significant change in the protease activity and PKC activity **[Table–2]**. Treatment of the cells with LTD_4 caused a marked increase in $[Ca^{2+}]i$ **[Table–3]**. Pretreatment of the cells with the intracellular Ca^{2+} chelator BAPTA-AM inhibited LTD_4 induced aprotinin sensitive protease activity; and PKC-α and $cPLA_2$ translocations and their activities in the cell membrane **[Table–1]**. Immunoblot study of the smooth muscle cell membrane, isolated from LTD_4 (10nM) treated condition, with polyclonal $cPLA_2$ antibody significantly increased its protein profile as evidenced by an increase in the 85-kDa immunoreactive protein band in the immunoblot **[Figure–1]**. Pretreatment of the cells with aprotinin, calphostin C, Go6976, and $AACOCF_3$ did not produce any change in the LTD_4 induced $cPLA_2$ immunoreactive protein profile in the membrane **[Figure 1]**.

Results in the parentheses indicate percent change over basal value. Protease activity is expressed as the change in absorbance at 410 nm/mg protein/30 min. PKC activity is expressed as pmol/mg protein/min; $cPLA_2$ activity is expressed as pmol AA/mg protein/min. [a]p<0.001 compared with basal condition; [b]p<0.01 compared with basal condition; [c]p<0.001 compared with LTD_4 treatment.

Table: 1. Effect of vitamin E, dithiothreitol (1 mM), aprotinin and BAPTA-AM on LTD_4 induced protease activity, PKC activity, $cPLA_2$ activity in bovine pulmonary artery smooth muscle cell membrane [Results are mean ± SE (n=4)]

Treatment	Protease activity	PKC activity	PLA_2 activity
Basal	0.22 ± 0.02	104 ± 9	0.92 ± 0.10
LTD_4 (10nM)	2.94 ± 0.18a (+1236)	898 ± 22a (+763)	6.87 ± 0.21a (+647)
Vitamin E (1 mM)	0.18 ± 0.02 (-18)	94 ± 8 (-10)	0.81 ± 0.06 (-12)
Vitamin E (1 mM) + LTD_4 (10nM)	0.20 ± 0.02c (-9)	99 ± 9c (-5)	0.84 ± 0.07c (-9)
DTT (1mM)	0.19 ± 0.02 (-14)	98 ± 8 (-6)	0.86 ± 0.08 (-7)
DTT (1mM) + LTD_4 (10nM)	0.24 ± 0.02c (-9)	102 ± 8c (-2)	0.89 ± 0.08c (-3)
Aprotinin (10□g/ml)	0.06 ± 0.008b (-73)	92 ± 7 (-12)	0.86 ± 0.05 (-7)
Aprotinin (10□g/ml) + LTD_4 (10nM)	0.08 ± 0.009c (-64)	96 ± 8c (-8)	0.88 ± 0.06c (-4)
BAPTA-AM (50 μM)	0.06 ± 0.008b (-73)	95 ± 8 (-9)	0.21 ± 0.02b (-77)
BAPTA-AM (50 μM) + LTD_4 (10nM)	0.07± 0.009c (-68)	98 ± 8c (-6)	0.24 ± 0.02c (-74)

Table: 2. Effect of different treatments on LTD_4 (10nM) induced protease activity, PKC activity, $cPLA_2$ activity in bovinepulmonary artery smooth muscle cell membrane [Results are mean ± SE (n=4)]

Treatment	Protease activity	PKC activity	$cPLA_2$ activity
Basal	0.22 ± 0.02	104 ± 9	0.92 ± 0.10
LTD_4 (10nM)	2.94 ± 0.18a (+1236)	898 ± 22a (+763)	6.87 ± 0.21a (+647)
$AACOCF_3$ (10 μM)	0.21 ± 0.02 (-5)	98 ± 8 (-6)	0.24 ± 0.02b (-74)
$AACOCF_3$ (10μM)+ LTD_4 (10nM)	2.92 ± 0.19a (+1227)	892 ± 29a (+758)	0.28 ± 0.02c (-70)
Bel (10 μM)	0.20 ± 0.02 (-9)	99 ± 9 (-5)	0.84 ± 0.06 (-9)
Bel (10 μM) + LTD_4 (10nM)	2.95 ± 0.16a (+1241)	896 ± 21a (+762)	6.86 ± 0.22a (+646)
Calphostin C (1 μM)	0.21 ± 0.02 (-5)	32 ± 4b (-69)	0.88 ± 0.06 (-4)
Calphostin C(1μM)+ LTD_4 (10nM)	2.91 ± 0.17a (+1223)	38 ± 4c (-63)	1.02 ± 0.08c (+11)
Go6976 (1 μM)	0.20 ± 0.02 (-9)	54 ± 5b (-48)	0.89 ± 0.07 (-3)
Go6976 (1 μM) + LTD_4 (10nM)	2.92 ± 0.19a (+1227)	64 ± 6c (-38)	1.08 ± 0.08c (+17)

Table 3: Effect of LTD_4 (10nM) treatment on $[Ca^{2+}]_i$, the cell membrane associated $cPLA_2$ activity and PKC activity in bovine pulmonary artery smooth muscle cells [Results are mean ± SE (n = 4)]

Condition	$[Ca^{2+}]_i$	Protease activity	PKC activity	$cPLA_2$ activity
Basal	164 ± 8	0.22 ± 0.02	104 ± 9	0.92 ± 0.10
LTD_4 (10nM)	1106 ± 32a (+574)	2.94 ± 0.18a (+1236)	898 ± 22a (+763)	6.87 ± 0.21a (+647)

EDITED BY – PROF. S.K. MAULIK; MD, PHD

RESEARCH

www.iioab.org

THE IIOAB JOURNAL

www.iioab.webs.com

THE IIOAB JOURNAL
ISSN: 0976-3104

www.iioab.org

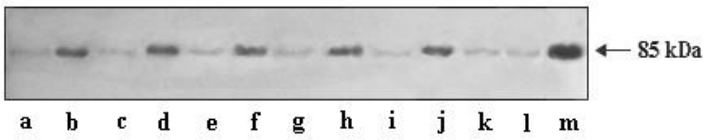

Fig: 1. Immunoblot study of the presence of immunoreactive cPLA$_2$ protein in cell membrane isolated from bovine pulmonary artery smooth muscle cells under different treatments. Lane a, basal condition; lane b, LTD_4 (10nM) treatment; lane c, aprotinin (10 µg/mL) treatment; lane d, aprotinin (10 µg/mL) treatment followed by addition of LTD_4 (10nM) ; lane e, calphostin C (1 µM) treatment; lane f, calphostin C (1 µM) treatment followed by addition of LTD_4 (10nM); lane g, Go6976 (1 µM); lane h, Go6976 (1 µM) + LTD_4 (10nM); lane i, $AACOCF_3$ (10µM) treatment; lane j, $AACOCF_3$ (10 µM) treatment followed by addition of LTD_4 (10nM); lane k, BAPTA-AM (50 µM) treatment; lane l, BAPTA-AM (50 µM) treatment followed by the addition of LTD_4 (10nM); lane m, standard cPLA$_2$.

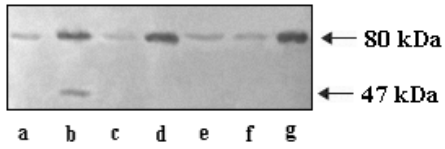

Fig: 2. Effect of different treatments on immunoreactive protein kinase Cα protein profile in the membrane isolated from bovine pulmonary artery smooth muscle cells. Lane a, basal condition; lane b, LTD_4 (10nM) treatment; lane c, aprotinin (10µg/mL) treatment; lane d, aprotinin (10µg/mL) treatment followed by addition of LTD_4 (10nM); lane e, BAPTA-AM (50 µM) treatment; lane f, BAPTA-AM (50 µM) treatment followed by addition of LTD_4 (10nM) ; lane g, standard protein kinase Cα.

Results in the parentheses indicate percent change over basal value. $[Ca^{2+}]_i$ is expressed in nM $Ca^{2+}/10^5$ cells. Protease activity is expressed as the change in absorbance at 410 nm/mg protein/30 min. PKC activity is expressed as pmol/mg protein/min; cPLA$_2$ activity is expressed as pmol AA/mg protein/min.

LTD_4 causes an increase in $[Ca^{2+}]_i$ in the smooth muscle cells **[Table–3]**. Since conventional PKCs (cPKCs) are activated by an increase in $[Ca^{2+}]_i$, we used polyclonal antibodies of conventional PKCs (α, β, and γ subtypes) in order to determine the exact PKC isoform(s) that has been translocated from cytosol to the cell membrane under exposure of the cells with LTD_4. Treatment of the cells with LTD_4 translocates the 80-kDa PKCα to the cell membrane **[Figure–2]**. No change in the immunoreactive band for β and γ subspecies of the cPKCs in the membrane were observed under LTD_4 stimulation in the immunoblot (results not shown). Thus, it appears that LTD_4 (10nM) causes translocation and activation of PKCα in the smooth muscle cell membrane **[Figure–2; Table–1]**. Under this condition, a low-molecular weight band (~47 kDa) along with the 80kDa immunoreactive protein profile was also observed **[Figure–2]**. The low molecular weight band (~47 kDa) in the immunoblot of the membrane fraction appears to be due to proteolytic cleavage of the 80-kDa PKCα isoform because pretreatment with aprotinin abolished the 47-kDa immunoreactive profile **[Figure–2]**. Pretreatment of the cells with Go6976 (1 µM), prevents LTD_4 induced increase in the PKC activity & cPLA$_2$ activity in the membrane **[Table– 2]**.

[IV] DISCUSSION

Our present studies suggest that LTD_4 caused stimulation of cPLA$_2$ activity is mediated by reactive free radicals (ROS) because pretreatment with vitamin E and dithiothreitol prevent LTD_4 induced increase in the enzyme activity **[Table–1]**. Two lines of evidence suggest that LTD_4 stimulates cPLA$_2$ activity in the membrane. First, LTD_4 increases the immunoreactive cPLA$_2$ protein content in the cell membrane **[Figure–1]**. And, secondly, the cPLA$_2$ inhibitor $AACOCF_3$, but not the $_i$PLA$_2$ inhibitor Bel, prevents LTD_4 induced cPLA$_2$ activity in the membrane **[Table–2]**. cPLA$_2$ was identified as a cytosolic protein in some type of cells and its activity has been shown to be regulated through Ca^{2+}-dependent translocation to the cell membrane [19]. Herein, we demonstrated that treatment of the cells with LTD_4 markedly increases cPLA$_2$ immunoreactive protein profile in the membrane. A pertinent question that may be asked at this stage is whether the increase in protease activity, PKC-α activity, and cPLA$_2$ activity in the smooth muscle cell membrane occur due to an increase in intracellular Ca^{2+} by LTD_4 The observed changes in the immunoreactive PKC-α and cPLA$_2$ protein profiles, and the generation of 47-kDa immunoreactive fragment of PKC-α with subsequent increase in cPLA$_2$ activity in the membrane under LTD_4 treatment to the cells appear to occur due to a marked increase in $[Ca^{2+}]_i$ Interestingly, pretreatment of the cells with aprotinin, calphostin C and $AACOCF_3$ could not reverse LTD_4 mediated increase in the immunoreactive cPLA$_2$ protein content in the cell membrane **[Figure–1]**. Previous study

EDITED BY – PROF. S.K. MAULIK; MD, PHD
RESEARCH
www.iioab.webs.com

THE IIOAB JOURNAL ISSN: 0976-3104

www.iioab.org

THE IIOAB JOURNAL

www.iioab.webs.com

suggested that mere translocation of $cPLA_2$ to the cell membrane does not accompany with activation of the enzyme [20]. It, therefore, seems conceivable that the $cPLA_2$ is exported from cytosol to the membrane upon treatment of the cells with LTD_4 and that this translocation of $cPLA_2$ to the membrane is a prerequisite for $cPLA_2$ activation in the cells.

Several lines of evidence suggest that an aprotinin sensitive protease plays an important role in activating PKC-α and subsequent activation of $cPLA_2$ activity in bovine pulmonary artery smooth muscle cells under LTD_4 triggered condition. First, the smooth muscle cell membrane exhibits an aprotinin-sensitive protease activity **[Table–1]**. Secondly, LTD_4 not only augments $cPLA_2$ activity and PKC-α activity but also dramatically increases an aprotinin-sensitive protease activity in the cell membrane **[Table–1]**. Thirdly, the protease inhibitor, aprotinin prevents LTD_4-mediated increase in the protease activity, PKC activity, and $cPLA_2$ activity in the smooth muscle cell membrane **[Table–1]**. Fourthly, treatment of the cells with LTD_4 causes translocation of 80-kDa PKCα to the membrane **[Figure–2]**. Under this condition, a low-molecular weight band (~47 kDa) along with the 80-kDa immunoreactive profile was also observed **[Figure–2]**. In some types of cells such as human fibroblast, human neutrophils and rat skeletal muscle cells, proteolytic activation of PKCα has been demonstrated [21, 22]. Herein, we found that pretreatment with aprotinin abolished the 47-kDa immunoreactive fragment. The 47-kDa immunoreactive fragment appears to be the active fragment of PKC-α. These four lines of evidence support our working hypothesis that an aprotinin-sensitive protease plays a pivotal role in activating PKC-α and subsequently stimulating $cPLA_2$ activity in the smooth muscle cell membrane under LTD_4 triggered condition. The mechanism by which LTD_4 derived ROS stimulates aprotinin sensitive protease is currently unknown. Previous reports that inactivation of endogenous protease inhibitors by oxidants causes an imbalance between protease and antiprotease with the resultant shift of the equilibrium towards protease [13]. Considering the fact that pretreatment of the cells with DTT inhibited LTD_4 induced increase in the protease activity, it seems conceivable that oxidants generated by LTD_4 cause redox modification by thiol exchange of cysteine residues of aprotinin and that may be an important mechanism of its inactivation resulting in the stimulation of the protease activity, which in turn activates PKC-α and $cPLA_2$ activity in the cell membrane. The target site of action of LTD_4 induced PKC-α remains to be determined. It could act directly on $cPLA_2$ or may act via PLA_2 activating or inhibiting proteins [23, 24] or may act via a pertussis toxin sensitive G protein [25].

[V] CONCLUSION

The present study suggest that (i) treatment of bovine pulmonary artery smooth muscle cells with LTD_4 causes an increase in $cPLA_2$ activity in the cell membrane through the involvement of reactive oxygen species; (ii) proteolytic activation of PKC-α by an aprotinin sensitive protease appears to be an important mechanism for optimum activation of $cPLA_2$ in the cell membrane during LTD_4 stimulation of the smooth muscle cells.

ACKNOWLEDGEMENT

Thanks are due to Prof. John R Michael, Department of Medicine, University of Utah, Salt Lake City, Utah, USA and Prof. Gerry Shaw, Department of Neurosciences, University of Florida, Gainesville, Florida, USA for their interest in our research. This article is dedicated to late Prof. Gail H. Gurtner, Departments of Physiology and Medicine, New York Medical College, Valhalla, New York, USA.

REFERENCES

[1] Chakraborti S, Gurtner GH, Michael JR. [1989] Oxidant-mediated activation of phospholipase A2 in pulmonary endothelium. *Am J Physio* 257: L430–L437.

[2] Farrukh I, Spannahake EW, Smith AM, et al. [1986] Leukotriene D4 increases pulmonary vascular permeability and pressure by different mechanisms in the rabbit. *Am Rev Respir Dis* 134: 229–232.

[3] Ravasi S, Citro S, Viviani B, et al. [2006] CysLT receptor induced airway smooth muscle cells proliferation requires ROS generation, EGF receptor transactivation and ERK1/2 phosphorylation. *Respir Res* 42: 1v18.

[4] Larfars G, Lantoine F, Devynck MA, et al. [1999] Activation of nitric oxide release and oxidative metabolism by leukotriene B_4, C_4, D_4 in human polymorphonuclear leukocytes. *Blood* 99:1399–1405.

[5] Hui Y, Chang Y, Smalera Jian W, et al. [2004] Directed vascular expression of human cysteinyl leukotriene 2 receptor modulates endothelial permeability and systemic blood pressure. *Circulation* 110: 3360–3366.

[6] Gurtner GH, Knoblauch A, Smith PS, et al. [1983] Oxidant and lipid induced pulmonary vasoconstriction mediated by arachidonic acid metabolites. *J Appl Physiol* 55: 949–954.

[7] Clark MA, Littlejohn D, Conway TM, et al. [1986] Leukotriene D_4 treatment of bovine aortic endothelial cells and murine smooth muscle cells in culture results in an increase in phospholipase A_2 activity. *J Biol Chem* 261: 10713–10718.

[8] Shan GX. [2003] Selective protein kinase C inhibitor and their applications. Current Drug Targets: *Cardiovascular & Haematological Disorders* 3: 301–307.

[9] Eto A, Akita Y, Saido TC, et al. [1995] The role of the calpain-calpastatin system in thyrotropin-releasing hormone-induced selective down-regulation of a protein kinase C isozyme, nPKC epsilon, in rat pituitary GH4C1 cells. *J Biol Chem* 270: 25115–25120.

[10] Hussain S, Assender JW, Bond M, et al. [2002] Activation of protein kinase C zeta is essential for cytokine-induced metalloproteinase-1, -3, and -9 secretion from rabbit smooth muscle cells and inhibits proliferation. *J Biol Chem* 277: 27345–27352.

[11] Seeger W, Wolf H, Graubert E, et al. [1983] Influence of aprotinin and gabexate mesylate on arachidonic acid release by the Ca-ionophore A23187 in the lung. *Adv Expt Med Biol* 156: 553–567.

EDITED BY – PROF. S.K. MAULIK; MD, PHD

RESEARCH

THE IIOAB JOURNAL ISSN: 0976-3104

[12] Hashimoto E, Yamamura H. [1989] Further studies on the ionic strength dependent proteolytic activation of protein kinase C in rat liver plasma membrane by endogenous trypsin-like protease. *J Biochem (Tokyo)* 106: 1041–1048.
[13] Chakraborti T, Ghosh SK, Michael JR, et al. [1996] Role of an aprotinin sensitive protease in the activation of Ca^{2+}ATPase by superoxide radical (O_2^-) in microsomes of pulmonary smooth muscle. *Biochem* J 317:885–890.
[14] Grynkiewicz G, Poenie M, Tsien RY. [1985] A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J Biol Chem* 260:3440–3450.
[15] Towbin H, Staehelin T, Gordon J. [1979] Electrophoretic transfer of proteins from polyscrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc Nat Acad Sci (USA)* 76: 4350–4354.
[16] Kitano T, Go M, Kikkawa U, et al. [1986] Assay and purification of protein kinase C. *In Methods in Enzymology* 124: 349–352.
[17] Smith PK, Krohn RI, Hermanson GT, et al. [1985] Measurement of protein using bicinchoninic acid. *Anal Biochem* 150:76–85.
[18] Daniel WW. [1978] A foundation for analysis in the health in sciences, chapter: Estimation. Biostatistics (Daniel WW, Ed.), John Wiley & Sons, *New York* p 121–157.
[19] Kramer RM, Sharp JD. [1997] Structure, function and regulation of Ca^{2+}-sensitive cytosolic phospholipase A_2. *FEBS Letters* 410,: 49–53.
[20] Persaud SJ, Jones PM, Roderigo-Milne HM, Buchan AM, Squires PE. [2003] Calcium-dependent translocation of cytosolic phospholipase A2 in pancreatic beta-cells. *Biochem Biophys Res Commun* 300: 889–893.
[21] Hong DH, Huan J, Ou BR, et al. [1995] Protein kinase C isoforms in muscle cells and their regulation by phorbol ester and calpain. *Biochim Biophys Acta* 1267: 45–54.
[22] Gilligan DM, Sarid R, Weese J. [2002] Adducin in platelets: Activation induced phosphorylation by PKC and proteolysis by calpain. *Blood* 99: 2418–2426.
[23] Clark MA, Chen MJ, Crooke ST, et al. [1988] Tumor necrosis factor induces phospholipase A2 activity and synthesis of a phospholipase A_2 activating protein in endothelial cells. *Biochem J* 250: 125–132.
[24] Blackwell GJ, Carnuccio R, DiRosa M, et al. [1980] Macrocortin: a polypeptide causing the anti-phospholipase A_2 effect of glucocorticoids. *Nature (Lond)* 287: 147–149.
[25] Chakraborti T, Das S, Chakraborti S. [2005] Proteolytic activation of protein kinase Cα by peroxynitrite in stimulating cytosolic phospholipase A_2 in pulmonary endothelium: Involvement of a pertussis toxin sensitive protein. *Biochemistry* 44: 5246–5257.

ABOUT AUTHORS

Dr. Sajal Chakraborti *is a Professor of Biochemistry, Department of Biochemistry & Biophysics, University of Kalyani, Kalyani 741235, West Bengal, India.*

Dr. Tapati Chakraborti *is an Associate Professor of Biochemistry, Department of Biochemistry & Biophysics,Uuniversity of Kalyani, Kalyani 741235, West Bengal, India.*

Md. Nur Alam *is a Research Scholar working with Prof. Sajal Chakraborti and Tapati chakrabori.*

Soni Shaikh *is a Research Scholar working with Prof. Sajal Chakraborti.*

Soumitra Roy *is a Research Scholar working with Prof. Sajal Chakraborti*

EDITED BY – PROF. S.K. MAULIK; MD, PHD

www.iioab.org THE IIOAB JOURNAL www.iioab.webs.com RESEARCH