Inhibition by fatty acids of cyclic AMP-dependent protein kinase activity in brush border membranes isolated from human placental vesicles

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1 The inhibitory effects of arachidonic acid (AA) and a number of structurally related fatty acids on cyclic AMP-dependent protein kinase activity have been investigated in brush border membranes (BBM) prepared from human placental vesicles.

2 BBM vesicles were characterized by electron microscopy and displayed enrichment of the appropriate marker enzymes, alkaline phosphatase and γ -glutamyltranspeptidase; BBM were prepared by vesicle lysis in hypotonic medium.

3 Cyclic AMP-dependent protein kinase (PKA) activity was measured in BBM. At 1 μ M, cyclic AMP stimulated a 4.2 ± 0.06 fold increase over basal levels of [³²P]-phosphate incorporation into the synthetic substrate kemptide and this effect was abolished by a selective PKA inhibitor. By use of synergistic pairs of site-selective cyclic AMP analogues, the kinase was identified as the type II enzyme.

4 Cyclic AMP-stimulated PKA activity was inhibited by 10 μ M AA and this effect was significantly enhanced by nordihydroguaiaretic acid (NDGA) + indomethacin (Indo), inhibitors of the lipoxygenase and cyclo-oxygenase pathways of AA metabolism respectively.

5 Oleic acid, elaidic acid, but not caprylic or palmitic acids, also significantly inhibited PKA activity and this effect was again enhanced by NDGA + Indo. While arachidonyl alcohol alone was not inhibitory, in the presence of the metabolic inhibitors a significant reduction in stimulated activity was observed.

6 The commercially available PKA type II holoenzyme (activated by cyclic AMP), but not the free catalytic subunit, was inhibitable by AA, oleic or elaidic acids.

7 These results suggest that PKA localized to the brush border membrane of human placental vesicles is inhibited by fatty acids which may compete with cyclic AMP for binding to the kinase regulatory subunit. The reported inhibition by fatty acids of cyclic AMP-dependent Cl⁻ secretion in epithelial cells may therefore be due in part to negative regulation of a Cl⁻ channel-associated PKA.

Keywords: Fatty acids; arachidonic acid; cyclic AMP-dependent protein kinase; human placenta; brush border membranes

Introduction

The regulation of chloride (Cl⁻) secretion by epithelial cells is an important process in fluid and electrolyte homeostasis. Transepithelial Cl⁻ movement in secretory epithelia involves entry via the Na⁺/K⁺/Cl⁻ cotransporter at the basolateral membrane and exit through Cl⁻ channels in the apical (brush border) membrane. These channels are regulated *inter alia* by adenosine 3':5'-cyclic monophosphate (cyclic AMP)-dependent protein kinase (PKA), protein kinase C, and calcium/calmodulin-dependent protein kinase (Welsh, 1990; Wagner *et al.*, 1991). Arachidonic acid (AA) and other unsaturated fatty acids have also been shown to regulate Cl⁻ channels and to inhibit the outwardly rectifying Cl⁻ channel in airway epithelia (Hwang *et al.*, 1990). The nature of this inhibition is unclear and it has been proposed that fatty acids interact with the channel or an associated protein to alter its function (Anderson & Welsh, 1990). In this study we provide evidence that PKA, localized to the brush border membrane of human placental vesicles, may constitute a target for the negative regulation of Cl⁻ channels by unsaturated fatty acids. We used brush border membranes prepared from placental vesicles, as opposed to whole cells, in our studies since we wished as far as possible to investigate direct effects of fatty acids on membrane PKA activity, free of regulation by intracellular second messenger systems, or by events occurring at the basolateral membrane. Human placental brush border membrane vesicles have previously been shown

to exhibit a Cl⁻ conductance (Dehecchi & Cabrini, 1988; Illsley *et al.*, 1988) which is inhibitable by AA (Faller & Ryan, 1992).

Methods

Preparation of brush border membrane vesicles

Term human placental tissue was obtained within 30 min of delivery and transported to the laboratory in a solution comprising equal volumes of Dulbecco's Modified Eagle's Medium (DMEM) and phosphate buffered saline (PBS, pH 7.4) at 4°C. Brush border membrane vesicles (BBMV) were prepared as described by Faller & Ryan (1992): the central portion between the maternal and foetal surfaces of the placenta was used, following removal of the maternal decidua. The maternal villous tissue was chopped into small pieces, washed in Buffer A (300 mM mannitol, 10 mM HEPES/Tris HCl, pH 7.0), further finely chopped and agitated in 300 ml Buffer A with a magnetic stirrer for 1 h at 4°C. Following removal of solid tissue by filtration through gauze, the filtrate was centrifuged at 1,000 g for 10 min, and the resulting supernatant recentrifuged at 86,000 g for 35 min. The pellet obtained was homogenized in 50 ml ice-cold Buffer A in a Dounce homogenizer and the resulting homogenate made 10 mM with respect to MgCl₂, stirred for 15 min at 4°C and allowed to stand for a further 15 min. The homogenate was then centrifuged at 5,000 g for 15 min and

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the resulting supernatant recentrifuged at 86,000 *g* for 35 min. Pellets were resuspended in Buffer A and aliquots snap frozen before storage in liquid nitrogen until required.

Characterization of BBMV

Electron microscopy of BBMV was carried out on samples fixed in phosphate buffer (pH 7.4) containing 2.5% glutaraldehyde, and post fixed in osmium tetroxide. Transmission electron micrographs were produced with a Philips 201 electron microscope. Scanning electron micrographs were produced with a JEOL JEM-200FX electron microscope.

Alkaline phosphatase (EC 3.1.3.1) activity was compared in placental homogenate and BBMV by the methods of Lansing *et al.* (1967). The enzyme substrate used was *p*-nitrophenylphosphate. The amount of *p*-nitrophenol produced was quantified by its absorption at 420 nm and was taken as an index of alkaline phosphatase activity, expressed as $\mu\text{mol } p\text{-nitrophenol formed mg}^{-1} \text{ protein min}^{-1}$.

γ -Glutamyltranspeptidase (EC 2.3.2.2) activity was also measured in homogenate and BBMV, according to Szasz (1969). The enzyme substrate was γ -glutamyl *p*-nitroanilide. The amount of *p*-nitroaniline produced by hydrolysis was quantified by its absorption at 405 nm and was taken as an index of γ GT activity, expressed as international units of enzyme activity $\text{mg}^{-1} \text{ protein min}^{-1}$.

Manipulation of BBMV for measurement of kinase activity

To ensure access of impermeant molecules to their intracellular targets, vesicles were resuspended in 75 mM phosphate buffer, pH 7.0, a procedure calculated to lyse the vesicles osmotically (Placchi *et al.*, 1991). The altered morphology of the resulting brush border membranes (BBM) was confirmed in a number of preparations by transmission electron microscopy.

Measurement of cyclic AMP-dependent protein kinase (PKA) activity

PKA (EC 2.7.1.37) activity was measured by an assay based on the transfer of the terminal phosphate of $\gamma\text{[}^{32}\text{P]}\text{-ATP}$ to the synthetic substrate kemptide (Giembycz & Diamond, 1990). Assays were carried out at 30°C in a final volume of 100 μl incubation mixture containing 60 μg protein, 'assay cocktail' (0.1 mM EGTA, 40 mM NaF, 1 mM dithiothreitol, 1 mM theophylline, 5 mM sodium arsenate), 10 μg kemptide, stimulators/inhibitors as appropriate, in 75 mM phosphate, pH 7.0. Following a 10 min preincubation period, the reaction was initiated by addition of 2 mM magnesium acetate + 1 mM $\gamma\text{[}^{32}\text{P]}\text{-ATP}$ ($1\text{--}2 \times 10^7$ c.p.m. mmol^{-1}). Assays were stopped after 20 min and processed by a modification of the method of Witt & Roskoski (1975): assay mixture was spotted onto P81 phosphocellulose ion exchange chromatography filter papers which were allowed to dry for 30 s and placed in a 75 mM phosphoric acid solution (10 ml/filter). Filters were then washed 3×10 min in acid, 1×5 min in methanol and ether. Incorporated radioactivity was determined by scintillation counting. Activities were expressed as pmol phosphate transferred $\text{mg}^{-1} \text{ protein min}^{-1}$.

Use of synergistic pairs of site selective cyclic AMP analogues

The two major isozymes of cyclic AMP-dependent protein kinase (PKA), type I and type II, are distinguished in terms of their regulatory subunits which provide the basis for isozyme classification (Lohmann & Walter, 1984). Each regulatory subunit in turn contains two different intrasubunit cyclic AMP binding sites, sites 1 and 2, which selectively bind certain cyclic AMP analogues (Beebe *et al.*, 1988). Binding of a cyclic nucleotide at one site stimulates binding at the other

site. When two cyclic AMP analogues, each selective for a different binding site, are added in combination to PKA, the enzyme is activated in a synergistic fashion. Since the cyclic AMP analogue specificity of site 1 is different for the two isozymes, the synergistic activation of type I and II isozymes may be distinguished. We used the method of Beebe *et al.* (1988) to identify the subtype of PKA present in BBM. Assay mixtures contained 60 μg BBM protein. The type I synergistic pair comprised 1.2 μM (8-(6-aminoethyl)amino-adenosine 3':5'-cyclic monophosphate (8AHA) and 10 nM N6 benzoyladenine 3':5'-cyclic monophosphate (N6BZL). The type II pair comprised 7 nM 8-thiomethyladenosine 3':5'-cyclic monophosphate (8-S-CH3) and 10 nM N6 benzoyladenine 3':5'-cyclic monophosphate. The assay conditions were identical to those described above for measurement of PKA activity.

Calculations: the basal activity ratio (activity in the absence, divided by activity in the presence of 1 μM cyclic AMP) was determined and this value was subtracted from the analogue-stimulated activity ratio. Results were expressed as the increment in activity ratio produced by the analogue.

Protein determination

This was carried out according to Lowry *et al.* (1951).

Statistical analysis

Measurements of PKA activities are presented as mean values $\pm$ s.e.mean of *n* experiments each performed in triplicate. Statistically significant differences where appropriate were determined by an unpaired Student's *t* test and differences were deemed significant if $P < 0.05$.

Materials

$\gamma\text{[}^{32}\text{P]}\text{-ATP}$ (> 10 Ci mmol^{-1}) was purchased from Amersham (U.K.). Kemptide, PKA type II inhibitor from bovine heart, 3':5'-cyclic AMP-dependent protein kinase from bovine heart, protein kinase catalytic subunit from bovine heart, 8-(6-aminoethyl)aminoadenosine 3':5'-cyclic monophosphate, N6-benzoyladenine 3':5'-cyclic monophosphate, 8-thiomethyladenosine 3':5'-cyclic monophosphate, arachidonic, oleic, elaidic, palmitic and caprylic acids, and arachidonol alcohol were all obtained from Sigma. P81 filter paper was obtained from Whatman. All other chemicals were of the highest purity commercially available.

Fatty acids and arachidonol alcohol were dissolved in ethanol and stored in the dark in aliquots, under an atmosphere of nitrogen at -20°C until required. The final concentration of ethanol in all assays was less than or equal to 0.1% at which concentration ethanol was without effect on PKA activity.

Results

Characterization of brush border membrane vesicles

Alkaline phosphatase and γ -glutamyl transpeptidase are two enzymes localized to the brush border membranes of epithelial cells. When activities of these enzymes were compared in placental homogenates and in BBMV, alkaline phosphatase and γ -glutamyl transpeptidase were enriched in BBMV by factors of 25 ± 4.6 and 30.5 ± 7.8 respectively ($n = 4$ in each case). Transmission and scanning electron micrographs showed the vesicles to be spherical, intact and to have the brush border directed outwards. When vesicles were subjected to hypotonic lysis, the characteristic spherical shape was lost and open fragmented structures indicative of membranes became apparent.

Identification of the type of kinase present in BBM

The presence of PKA activity in BBM was first established. Basal activity was stimulated 4.2 ± 0.06 fold ($n = 4$) by $1 \mu\text{M}$ cyclic AMP. This stimulation was abolished by the cyclic AMP-dependent protein kinase inhibitor (Walsh inhibitor) PKI ($5 \mu\text{g ml}^{-1}$) as shown in Figure 1. PKI (a 75 amino acid protein isolated from rabbit skeletal muscle) inactivates the catalytic subunit of the kinase by binding to its protein-substrate site and has been reported to exhibit an extremely high degree of specificity for PKA (Walsh & Glass, 1991). An additional characteristic of PKA is its susceptibility to inhibition by Ca^{2+} and in BBM, stimulated PKA activity was reduced in the presence of 2 mM CaCl_2 (Figure 1). We next examined the effects of synergistic pairs of site-selective cyclic AMP analogues on PKA activity and the results obtained are summarized in Figure 2 for the type II directed analogue pair (N6BZL + 8-S-CH3). As can be seen, the increments in activity ratio for N6BZL and 8-S-CH3 were 0.03 and 0.13 respectively. An additive effect of these analogues would therefore have yielded a ratio of 0.16. However the change in activity ratio observed was 0.58, yielding a synergism quotient (ratio between observed and expected responses) of 3.6. No synergism was observed with the type I-directed pair (N6BZL + 8AHA, data not shown). Thus since the enzyme was synergistically activated by the type II-directed analogue pair only, it is likely to be type II.

Effect of arachidonic acid on cyclic AMP-dependent protein kinase activity

Cyclic AMP-stimulated PKA activity was inhibited by $10 \mu\text{M}$ arachidonic acid (AA; 20:4, *cis*-5,8,11,14) and this inhibition was significantly enhanced in the presence of a combination of nordihydroguaiaretic acid (NDGA) and indomethacin (Indo) (both $5 \mu\text{M}$), which are inhibitors of the lipoxygenase and cyclo-oxygenase pathways of AA metabolism respectively. NDGA + Indo alone did not inhibit cyclic AMP-stimulated PKA activity (results summarized in Figure 3). The enhanced effect in the presence of these inhibitors would suggest that the relevant enzymes are present in BBM (particulate fractions isolated from human placental villous tissue have recently been shown to possess lipoxygenase activity, see Joseph *et al.*, 1993). In separate experiments, no reduction of cyclic AMP stimulated activity was seen with the AA metabolites prostaglandin E_2 (PGE_2), $\text{PGF}_{2\alpha}$, 5-HPETE (each at $10 \mu\text{M}$) or leukotriene C_4 ($24 \mu\text{M}$) (data not shown).

Figure 1 PKA activity in brush border membranes and its stimulation by cyclic AMP ($1 \mu\text{M}$) alone and in the presence of PKI ($5 \mu\text{g ml}^{-1}$) or CaCl_2 (2 mM). Data represent the mean $\pm$ s.e. mean of 4 experiments performed in triplicate. Results are expressed as phosphate transferred ($\text{pmol min}^{-1} \text{mg}^{-1} \text{protein}$). Asterisks indicate significant differences between values linked by horizontal bars. * $P < 0.05$.

Effects of analogues on stimulated PKA activity

Both oleic acid ($18:1$, *cis*-9) and its *trans* isomer, elaidic acid ($18:1$, *trans*-9), each at a concentration of $10 \mu\text{M}$, significantly inhibited PKA activity in BBM and as with AA, the inhibitory effects of these analogues were enhanced in the presence of NDGA + Indo (see Figure 4). In contrast however, the saturated fatty acids palmitic acid ($16:0$) and caprylic acid ($8:0$), did not affect cyclic AMP-stimulated PKA activity at this concentration in the presence or absence of inhibitors and while arachidonyl alcohol alone ($10 \mu\text{M}$)

Figure 2 Synergism of cyclic AMP-dependent protein kinase activation in brush border membranes using a type II directed analogue pair. Membranes were incubated with 10 nM N^6 -benzoyl-cyclic AMP (N6BZL, open columns), 7 nM 8-thiomethyl-cyclic AMP (8-S-CH₃, hatched columns) or a combination of both (solid columns). The basal activity ratio determined in the absence and presence of $1 \mu\text{M}$ cyclic AMP was 0.08 and was subtracted from all other values. Results are expressed as the increment in activity ratio.

Figure 3 Effects of arachidonic acid (AA, $10 \mu\text{M}$) on cyclic AMP stimulated PKA activity in brush border membranes in the absence and presence of inhibitors of AA metabolism (NDGA, indomethacin, $5 \mu\text{M}$). Data represent the mean $\pm$ s.e. mean of 3 experiments each performed in triplicate. Results are expressed as % of cyclic AMP signal. Asterisks indicate significant differences between values linked by horizontal bars: * $P < 0.005$.

was also without effect, in the presence of NDGA + Indo significant reduction of stimulated activity was observed (Figure 5).

Effects of fatty acids on cyclic AMP-stimulated holoenzyme

With the objective of identifying the site(s) at which AA-mediated inhibition of PKA had occurred, inhibitory effects

Figure 4 Effects of oleic and elaidic acids (10 μ M) on cyclic AMP stimulated PKA activity in brush border membranes in the absence and presence of inhibitors of fatty acid metabolism (NDGA, indomethacin, 5 μ M). Data are expressed as the mean $\pm$ s.e. mean of 3 experiments performed in triplicate. Results are expressed as % of cyclic AMP signal. Asterisks indicate significant differences between values linked by horizontal bars: * P < 0.05.

Figure 5 Effects of caprylic and palmitic acids and arachidonyl alcohol (10 μ M) both in the presence and absence of inhibitors of fatty acid metabolism (NDGA, indomethacin, 5 μ M). Data represent the mean $\pm$ s.e. mean of 3 experiments performed in triplicate. Results are expressed as phosphate transferred (pmol min⁻¹ mg⁻¹ protein). Asterisks indicate significant differences between values linked by horizontal bars: * P < 0.025.

on stimulated activity of the commercially available type II holoenzyme were investigated. As can be seen in Figure 6, AA, oleic and elaidic acids again significantly lowered the cyclic AMP signal, while the saturated fatty acids palmitic and caprylic acid were again without effect. In these experiments 'basal' activity of the holoenzyme was rather high and PKI reduced the cyclic AMP signal to below 'basal' levels. This probably reflected some contamination of the holoenzyme by free catalytic subunit (CS), the activity of which would be independent of cyclic AMP, yet inhibitable by PKI.

Effect of AA on activity of the PKA catalytic subunit

Activity of the free catalytic subunit was significantly reduced by PKI, whereas AA at a concentration of 10 μ M was without effect (Figure 7).

Figure 6 Effects of various fatty acids (10 μ M) on cyclic AMP-stimulated activity of the PKA holoenzyme. Cyclic AMP (cAMP, 1 μ M) was present in all cases except where basal activity was measured. Abbreviations: arachidonic acid (AA), oleic acid (OA), elaidic acid (EA), palmitic acid (PA), caprylic acid (CA) and arachidonyl alcohol (A Alc). Data represent the mean $\pm$ s.e. mean of 3 experiments performed in triplicate. Results are expressed as phosphate transferred (nmol min⁻¹ mg⁻¹ protein). Asterisks indicate significant differences between values linked by horizontal bars: * P < 0.0005; ** P < 0.005 and *** P < 0.01.

Figure 7 Effects of PKI (5 μ g ml⁻¹) and arachidonic acid (AA, 10 μ M) on the activity of the PKA catalytic subunit type II from bovine heart. Data represent the mean $\pm$ s.e. mean of 3 experiments performed in triplicate. Results are expressed as phosphate transferred (nmol min⁻¹ mg⁻¹ protein). Asterisks indicate significant differences between values linked by horizontal bars: * P < 0.05.

Discussion

This study demonstrates for the first time, the inhibition of cyclic AMP-dependent protein kinase activity in human placental brush border membranes by unsaturated fatty acids. Though PKA-regulated Cl^- channel activity has been investigated in a number of such membrane systems there is not general agreement on whether the enzyme is membrane-associated in the vesicle preparation. Thus, while PKA was found to be associated with rabbit and canine cortical renal brush-border vesicles (Hammerman & Hruska, 1982; Weinmann *et al.*, 1987), it was necessary to add exogenous PKA to mouse renal medullary membranes (Reeves *et al.*, 1989) and to a human placental vesicle preparation used to investigate regulation of Cl^- transport (Placchi *et al.*, 1991). While the variability in expression of a membrane-associated kinase may simply reflect differences in the methods of vesicle preparation, it may also be due to the presence of different PKA isozymes in the various tissues. It has been proposed that types I and II are predominantly cytosolic and membrane-bound respectively (Lohmann & Walter, 1984) and therefore if a particular cell type expressed only the soluble type I enzyme, this would not be retained in the vesicle preparation unless activation/translocation had occurred during vesicle isolation. Since the kinase identified in the present study (using synergistic pairs of site-selective cyclic AMP analogues) appears to be type II, its membrane localization is to be expected. We would further propose that the enzyme is localized to the cytosolic surface of BBMV prior to lysis since there is evidence for the existence of proteinases on the outer surface of BBMV, which selectively degrade cyclic AMP-stimulated ectokinases in the course of vesicle isolation (De Jonge *et al.*, 1987).

AA inhibited PKA activity in our system at a concentration (10 μM) previously shown to inhibit Cl^- channel activity in airway epithelial cells (Anderson & Welsh, 1990; Hwang *et al.*, 1990; Faller & Ryan, 1992). A direct role for AA in PKA inhibition is supported by (i) the enhanced effect observed in the presence of inhibitors of metabolism by the lipoxygenase and cyclo-oxygenase pathways (Indo + NDGA) and (ii) by the lack of inhibition in the presence of the AA metabolites PGE_2 , $\text{PGF}_{2\alpha}$, leukotriene C_4 or 5-HPETE. Indeed AA has been reported to have stimulatory effects on Cl^- secretion via the production of metabolites capable of increasing cyclic AMP levels (Eling *et al.*, 1986). Furthermore in a study using dog tracheal epithelium, an AA-mediated increase in Cl^- secretion was converted to a reduction of secretion by the addition of inhibitors of AA metabolism (Mochizuki *et al.*, 1992). Direct stimulation by AA of epithelial Cl^- secretion has however also been reported recently in the T84 colonic epithelial cell line (Barrett & Bigby, 1993). These authors have suggested that AA may be stimulating Cl^- secretion via channels with conducting properties particular to intestinal epithelia.

To determine whether the inhibition of PKA activity in BBM was specific to AA, the effects of a number of structurally related analogues were examined. The lack of effect of caprylic and palmitic acids observed would agree with the specificity of Cl^- channel inhibition by fatty acids previously documented (Anderson & Welsh, 1990; Hwang *et al.*, 1990; Faller & Ryan, 1992), as would the inhibition obtained with oleic acid. However, in contrast to the findings of Faller & Ryan (1992), we obtained significant inhibition in our system with elaidic acid and with arachidonyl alcohol in the presence of NDGA + Indo. (The latter effect may be due to conversion of the alcohol to a substrate otherwise rapidly metabolized by cyclo-oxygenase or lipoxygenase.)

From our overall results it could be argued that the inhibition of PKA activity by fatty acids in this system is relatively non-specific and that the minimum structural requirement is a monounsaturated 18-carbon chain compound. We would propose an alternative model, namely one whereby all of the 'active' analogues may be converted to AA or a similar

polyunsaturated acid by a series of metabolic transformations involving desaturases. These enzymes are known to catalyse the production of polyunsaturated fatty acids from oleic acid (Gurr & James, 1980). Additional evidence for a common fatty acid mediator of PKA inhibition is suggested by the enhanced effect of all unsaturated compounds tested in the presence of NDGA + Indo, despite the fact that oleic and elaidic acids are not substrates for cyclo-oxygenase or lipoxygenase enzymes. In this model therefore, inhibitory activity would not be dependent on an all *cis*-unsaturated structure (elaidic acid was inhibitory) and so a general membrane lipid-altering effect characteristic of *cis*-unsaturated fatty acids (Klausner *et al.*, 1980) would be unlikely to account for the inhibition of PKA.

Fatty acid inhibition of PKA activity may involve binding to the regulatory subunit of the enzyme, since the commercially available PKA type II holoenzyme, but not the free catalytic unit, was inhibitable by unsaturated fatty acids under our assay conditions. It may be therefore that fatty acids act as negative regulators of cyclic AMP binding to the regulatory subunit of PKA.

The ability of unsaturated fatty acids to inhibit specifically a protein kinase has recently been reported in a prokaryotic system (Strauch *et al.*, 1992). These authors demonstrated an inhibition of the prokaryotic kinase Kin A *in vitro* by the *cis*-unsaturated linoleic, arachidonic and oleic acids. No inhibition of kinase activity by the saturated caprylic and palmitic acids was observed. However in contrast to our results, Strauch *et al.* (1992) reported that the *trans*-unsaturated fatty acid elaidic acid did not inhibit kinase activity.

The possibility of a physiological or pathophysiological role for AA or a related substance, in modulation of epithelial Cl^- secretion, has long been a matter for speculation. It has recently been demonstrated that cytosolic extracts purified from Cl^- secreting epithelial cells block outwardly rectifying Cl^- channels and although the exact nature of the inhibitor is unknown, the presence of fatty acids in these extracts has been suggested (Kunzelmann *et al.*, 1991). These authors have also speculated that in cystic fibrosis (CF), where epithelial cyclic AMP-regulated Cl^- transport is defective, elevated concentrations of this inhibitor may be present. Elevations in bradykinin-stimulated AA release have recently been reported in a CF epithelial cell line expressing the major deltaF508 mutation (Levistre *et al.*, 1993), though in this case it is not thought that the increases in AA produced are responsible for impaired regulation of Cl^- transport. Firm evidence has yet to be produced therefore, for a pathophysiological role for altered AA production in modulation of epithelial Cl^- transport.

In summary, this study has shown that a cyclic AMP-dependent protein kinase localized to the brush border membrane of human placental vesicles is subject to negative regulation by fatty acids with a selectivity comparable to that for Cl^- channel inhibition. We propose that the latter inhibition is mediated at least in part indirectly, via binding of fatty acids to the regulatory subunit of PKA which could then interact with a Cl^- channel.

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