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European Journal of Pharmacology 348 (1998) 297–304

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Fenspiride inhibits histamine-induced responses in a lung epithelial cell line

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Received 12 January 1998; revised 17 February 1998; accepted 20 February 1998

Abstract

Using the human lung epithelial WI26VA4 cell line, we investigated the capacity of fenspiride, an anti-inflammatory drug with anti-bronchoconstrictor properties, to interfere with histamine-induced intracellular Ca^{2+} increase and eicosanoid formation. Histamine and a histamine H_1 receptor agonist elicited a rapid and transient intracellular Ca^{2+} increase (0–60 s) in fluo 3-loaded WI26VA4 cells. This response was antagonized by the histamine H_1 receptor antagonist, diphenhydramine, the histamine H_2 receptor antagonist, cimetidine, having no effect. Fenspiride (10^{-7}–10^{-5} M) inhibited the histamine H_1 receptor-induced Ca^{2+} increase. In addition, histamine induced a biphasic increase in arachidonic acid release. The initial rise (0–30 s), a rapid and transient arachidonic acid release, was responsible for the histamine-induced intracellular Ca^{2+} increase. In the second phase release (15–60 min), a sustained arachidonic acid release appeared to be associated with the formation of cyclooxygenase and lipoxygenase metabolites. Fenspiride (10^{-5} M) abolished both phases of histamine-induced arachidonic acid release. These results suggest that anti-inflammatory and antibronchoconstrictor properties of fenspiride may result from the inhibition of these effects of histamine.

Keywords: Fenspiride; Histamine; Lung epithelial cell line; Ca^{2+}; Phospholipase A_2; Eicosanoid

1. Introduction

Fenspiride is a non-steroidal agent with anti-bronchoconstrictor properties (Advenier, 1988). It exerts an anti-inflammatory effect that is attributed to inhibition of phospholipase A_2 activity and subsequent release of eicosanoids (De Castro et al., 1995; Carré et al., 1991). It also has been shown that fenspiride may influence histamine-induced bronchoconstriction and airway allergic responses (Evrard et al., 1991; Lima et al., 1988).

Airway epithelial cells that may produce inflammatory mediators are often implicated in the initiation and the continuation of inflammation of airways (Levine et al., 1995). Histamine released by mast cells during airway allergic diseases (Jarjour et al., 1991; Djukanovic et al., 1990) increases intracellular Ca^{2+} concentration and the production of various inflammatory mediators, such as eicosanoids, in airway epithelial cells (Harris and Hanrahan, 1993; Vignola et al., 1993; Noah et al., 1991). The signal transduction mediated by the histamine H_1 receptor, which belongs to the superfamily of receptors coupled to G proteins, involves phospholipase C and/or phospholipase A_2 pathways (Arrang et al., 1995; Leurs et al., 1995; Murayama et al., 1990).

Among the active mediators released by epithelial cells in the lung, eicosanoids play an important role in the pathogenesis of airway inflammation (Holtzman, 1991). Eicosanoids are metabolites of arachidonic acid oxygenation by two main groups of enzymes, cyclooxygenases and lipoxygenases (Goetzl et al., 1995). Arachidonic acid liberated by phospholipase A_2, that hydrolyzes the *sn*-2-ester bond of phospholipids, is metabolized by cyclooxygenases to prostaglandins and thromboxanes, by 5-lipoxygenase to leukotrienes and by 5- or 15-lipoxygenases to 5- or 15-hydroxyeicosatetraenoic acid. Eicosanoids may regulate com-

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plex processes of cellular differentiation, migration and interactions through specific receptors (Goetzl et al., 1995; Holtzman, 1991). Arachidonic acid and its metabolites may act as second messengers, inducing intracellular Ca^{2+} increase (Tsunoda et al., 1996; Ramanadham et al., 1992; Axelrod et al., 1988). The histamine H_1 receptor has been shown to be coupled to Ca^{2+} influx via arachidonic acid release in DDT_1 MF-2 smooth muscle cells (Van der Zee et al., 1995).

In this study, we investigated the capacity of fenspiride to interfere with the histamine H_1 receptor-induced intracellular Ca^{2+} increase and eicosanoid formation in the SV40/adenovirus-transformed human lung epithelial WI26VA4 cell line. Histamine, via the histamine H_1 receptor, elicited a biphasic increase in arachidonic acid release. We show that the first phase, a rapid and transient arachidonic acid release, acts as a second messenger to mobilize Ca^{2+} in response to histamine. Fenspiride inhibited the histamine-induced arachidonic release and Ca^{2+} increase. We also provide evidence that the anti-histamine H_1 receptor properties of fenspiride follow from the inhibition of the second phase of arachidonic acid release and of subsequent eicosanoid formation in WI26VA4 cells.

2. Materials and methods

2.1. Materials

Histamine, diphenhydramine, lanthanum chloride, *p*-bromophenacylbromide and neomycin were obtained from Sigma (France), dimaprit and cimetidine from ICN biomedicals (France), 2-(2-aminoethyl)-pyridine from Aldrich (France), 1-(6-((17β-3-methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl)-1H-pyrrole-2,5-dione (U-73122) and TMB8 from Tebu (France), arachidonyl trifluoromethyl ketone ($AACOCF_3$) and isotetrandrine from Calbiochem (France), [^{3}H]arachidonic acid (1 mCi/ml) from Isotopchim (France), Fluo 3-AM (AcetoxyMethyl) from Interchim (France) and fenspiride from Servier (France). All products for culture were purchased from Gibco products (France).

2.2. Cell line

The human lung epithelial cell line, WI26VA4, was purchased from the European Collection of Animal Cell Cultures (ECACC, Porton Down). WI26VA4 cells were grown on 75 cm^2 tissue culture flasks in Eagle's minimum essential medium (EMEM) with Hank's balanced salt solution supplemented with 10% foetal calf serum, 2% non-essential amino acids, 2% vitamins and penicillin/streptomycin (500 000 UI–0.5 g).

2.3. Measurement of intracellular Ca^{2+} concentration

Intracellular Ca^{2+} concentration was measured in single cells by means of a video digital microscopy technique, using the fluorescent probe Fluo 3-AM as described previously (Sozzani et al., 1995). Briefly, WI26VA4 cells (2×10^4 cells) were plated into organ tissue culture dishes (60×15 mm, center well), and adherent cells were loaded with 7.5×10^{-6} M Fluo 3-AM for 1 h at 37°C. The cells were then washed twice with Hank's balanced salt solution (HBSS). The time course of the changes in the intracytosolic Ca^{2+} level was recorded every 2 s for a total of 2 or 10 min after addition of stimulating substances. In parallel assays, the cells were preincubated for 10 min with agonists or inhibitors before the addition of stimulating substances. A23187 (10^{-5} M) was added at the end of each experiment to assess the response with maximum intensity. The cells were visualized with an inverted microscope (Nikon Diaphot 300). The light source was a xenon lamp XBO 100 W (Osram, Munich, Germany). Excitation (488 nm) and emission (525 nm) wavelengths were selected with a XF23 filter block (Nikon). They were acquired by an intensified camera LHESA, LH 5038 (Cergy Pontoise, France). The images were digitized, and fluorescence was analyzed using the Imstar Starwise/Fluo Software System (Paris, France).

2.4. Determination of arachidonic acid and eicosanoid release

WI26VA4 cells (10^6 cells) were incubated into 6-well culture plates for 24 h with 2 μCi of [^{3}H]arachidonic acid in 2 ml EMEM. After incubation, the supernatant was removed, and the labelled cells were washed twice with 2 ml EMEM, and once with 2 ml phosphate buffer saline (PBS). The effect of histamine on arachidonic acid metabolism was then studied after short and long periods of time on labelled cells incubated at 37°C in an atmosphere of 5% CO_2.

Since the involvement of arachidonic acid as second messenger was being investigated, the reaction induced by histamine was stopped by the addition of 1 ml cold methanol–1% HCl in the short-duration experiments. The supernatants were collected, and the cell monolayer was scraped off and added to the supernatants, where many cells were found after addition of methanol–1% HCl. The supernatants were then extracted with chloroform, which allows efficient extraction of cell lipids (M'Rini-Puel et al., 1993). Briefly, the chloroform phase containing lipids, free arachidonic acid and metabolites of arachidonic acid was collected. These samples were then evaporated to dryness under nitrogen. The residues were dissolved in 80 μl of chloroform and applied to silica gel LK6DF Whatman, which had been activated previously (1 h at 100°C).

When the longer experiments were finished, given the high concentration of free [^{3}H]arachidonic acid in the culture medium compared to free [^{3}H]arachidonic acid in the cells, only the culture medium and the 1 ml EMEM used to wash cells were collected and centrifuged at $3000 \times g$ for 10 min at 4°C. The arachidonic acid and various arachidonic acid oxygenation products released by the cells into the culture medium were extracted by reversed phase extraction, and analysed by thin layer chromatography as previously described (M'Rini-Puel et al., 1993). Briefly, the supernatants were acidified (pH 5) with 1 M HCl and extracted by chromatography on Sep-Pak C18 columns (Waters). Arachidonic acid metabolites were eluted with methanol (5 ml) and evaporated to dryness under nitrogen. This procedure was very efficient for extracting arachidonic acid metabolites: approximately 95% extraction was reached. The residue was dissolved in 80 μl of CH_3OH and applied to silica gel LK6DF Whatman, which had been previously activated (1 h at 100°C).

The solvent system used to separate lipids, arachidonic acid and metabolites of arachidonic acid was, in both cases, the organic phase of ethyl acetate/water/isooctane/acetic acid (110:100:50:20 v/v). The radioactive metabolites were identified with a Berthold thin layer chromatography scanner.

2.5. Statistical analysis

For each arachidonic acid and eicosanoid release experiment, the results were expressed as means ± S.E.M. Statistical analysis was performed using a one-way analysis of variance (ANOVA), and the multiple comparisons of each treatment were evaluated by application of the Scheffe *F*-test.

3. Results

3.1. Histamine-induced intracellular Ca^{2+} increase via H_1 receptor in WI26VA4 cells

Histamine stimulation (10^{-4} M) of Fluo 3-loaded WI26VA4 cells caused a rapid and transient increase in the intracellular Ca^{2+} level (Fig. 1). This increase reached its maximum after 16 s and returned to the baseline after 45–60 s. To investigate the histamine receptors that are involved in the histamine-induced intracellular Ca^{2+} increase, cells were preincubated for 10 min with 10^{-4} M diphenhydramine, a histamine H_1 receptor antagonist, or 10^{-4} M cimetidine, a histamine H_2 receptor antagonist (Fig. 1A). Diphenhydramine blocked the increase of intracellular Ca^{2+} produced by histamine, whereas cimetidine had no effect on the stimulation of WI26VA4 cells. To confirm that the histamine H_1 receptor was involved in the modification of the Ca^{2+} level by histamine, we used a histamine H_1 receptor agonist, 2-(2-aminoethyl)-pyridine,

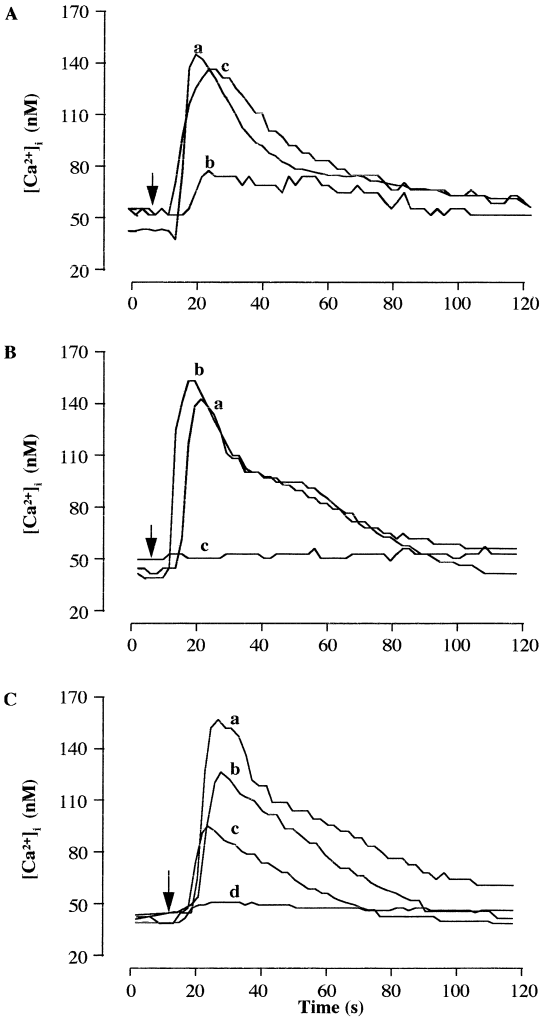

Fig. 1. Interference of fenspiride with histamine-induced intracellular Ca^{2+} increase. (A) Fluo 3-loaded WI26VA4 cells were stimulated (arrow) by histamine (10^{-4} M) without (a) or after preincubation with diphenhydramine (10^{-4} M, b), a histamine H_1 receptor antagonist, or cimetidine (10^{-4} M, c), a histamine H_2 receptor antagonist. (B) Fluo 3-loaded WI26VA4 cells were stimulated (arrow) with histamine (10^{-4} M, a), or with 2-(2-aminoethyl)pyridine (10^{-4} M, b), a histamine H_1 receptor agonist, or with dimaprit (10^{-4} M, c), a histamine H_2 receptor agonist. (C) Fluo 3-loaded WI26VA4 cells were stimulated (arrow) with histamine (10^{-4} M) without (a) or after preincubation with different concentrations (10^{-7} M, b; 10^{-6} M, c; 10^{-5} M, d) of fenspiride. All traces are representative of at least three separate experiments.

and a histamine H_2 receptor agonist, dimaprit (Fig. 1B). Like histamine, 10^{-4} M 2-(2-aminoethyl)-pyridine caused a rapid and transient increase in intracellular Ca^{2+} concentration, whereas 10^{-4} M dimaprit did not affect the Ca^{2+} level. After pretreatment with 10^{-4} M diphenhydramine, 2-(2-aminoethyl)-pyridine had no effect on the Ca^{2+} level of WI26VA4 cells (data not shown). We therefore investigated the ability of fenspiride to interfere with histamine H_1 receptor-induced intracellular Ca^{2+} increase. Fenspiride dose dependently inhibited the Ca^{2+} response to histamine (Fig. 1C). Fenspiride inhibition reached a maximum at 10^{-5} M.

3.2. Involvement of phospholipase A_2 in histamine-induced intracellular Ca^{2+} increase

To explore the mechanism of fenspiride inhibition, we studied the signal transduction pathway coupled to the histamine H_1 receptor and which was involved in histamine-induced intracellular Ca^{2+} increase. Histamine H_1 receptor activation generally leads to inositol lipids breakdown and inositol-dependent intracellular Ca^{2+} changes (Arrang et al., 1995). As inositol release depends on phospholipase C activity, we tested the capacity of two phospholipase C inhibitors, neomycine and U-73122, to abolish the Ca^{2+} response to histamine in WI26VA4 cells. Neither 5×10^{-4} M neomycine nor 10^{-6} M U-73122, both incubated 10 min before histamine stimulation, abolished the intracellular Ca^{2+} increase (Fig. 2A).

The role of phospholipase A_2 and the role of the arachidonic acid pathway in the histamine-induced Ca^{2+} increase in WI26VA4 cells were explored. Histamine failed to increase the intracellular Ca^{2+} level when cells were preincubated with 10^{-5} M *p*-bromophenacylbromide, a phospholipase A_2 inhibitor (Fig. 2B). We then tested a second inhibitor of phospholipase A_2, the trifluoromethylated analogue of arachidonic acid, $AACOCF_3$. Histamine stimulation in the presence of 10^{-5} M inhibitor did not lead to intracellular Ca^{2+} changes (Fig. 2B). Isotetrandrine, a specific phospholipase A_2-coupled G protein inhibitor (Shuttleworth, 1996; Tsunoda and Owyang, 1995; Akiba et al., 1992), also inhibited the histamine-induced intracellular Ca^{2+} increase (30 ± 4 nM with isotetrandrine vs. 127 ± 10 nM without isotetrandrine, $n = 6$).

3.3. Involvement of arachidonic acid release in histamine-induced intracellular Ca^{2+} increase

Since phospholipase A_2 seems to be involved in the intracellular Ca^{2+} increase induced by histamine, we measured the time course of arachidonic acid release and eicosanoid production induced by histamine in [^{3}H]arachidonic acid-labelled WI26VA4 cells. The results showed that histamine induced a biphasic increase in [^{3}H]arachidonic acid release (Fig. 3A and Fig. 4A). The rapid first phase occurred between 0 and 30 s and reached a maximum at 15 s (Fig. 3A). Thin-layer chromatography showed that the peak at 15 s was composed of only arachidonic acid and not arachidonic acid metabolites. No significant peak of arachidonic acid metabolites appeared until after 3 min. Both phospholipase A_2 inhibitors, *p*-bromophenacylbromide and $AACOCF_3$, which inhibited histamine-induced intracellular Ca^{2+} increase, also prevented arachidonic acid release (68 ± 14% and 61 ± 8% of inhibition, $n = 3$, respectively).

We then examined the effect of arachidonic acid on the Ca^{2+} increase in WI26VA4 cells. Exposure of Fluo-3-loaded WI26VA4 cells to arachidonic acid (10^{-6} M, 10^{-5}

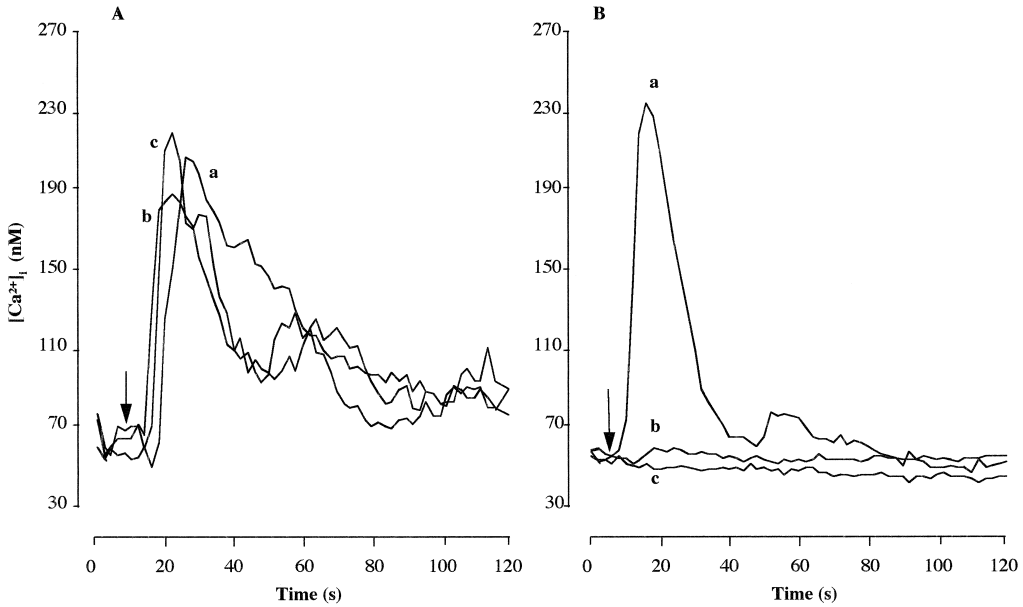

Fig. 2. Involvement of phospholipase C and phospholipase A_2 in the histamine-induced intracellular Ca^{2+} increase. (A) Fluo 3-loaded WI26VA4 cells were stimulated (arrow) with histamine (10^{-4} M) without (a) or after preincubation with two distinct phospholipase C inhibitors, neomycine (5×10^{-4} M, b) and U-73122 (10^{-6} M, c). (B) Fluo 3-loaded WI26VA4 cells were stimulated (arrow) with histamine (10^{-4} M) without (a) or after preincubation with two distinct phospholipase A_2 inhibitors, *p*-bromophenacylbromide (10^{-5} M, b) and $AACOCF_3$ (10^{-5} M, c). All traces are representative of at least three separate experiments.

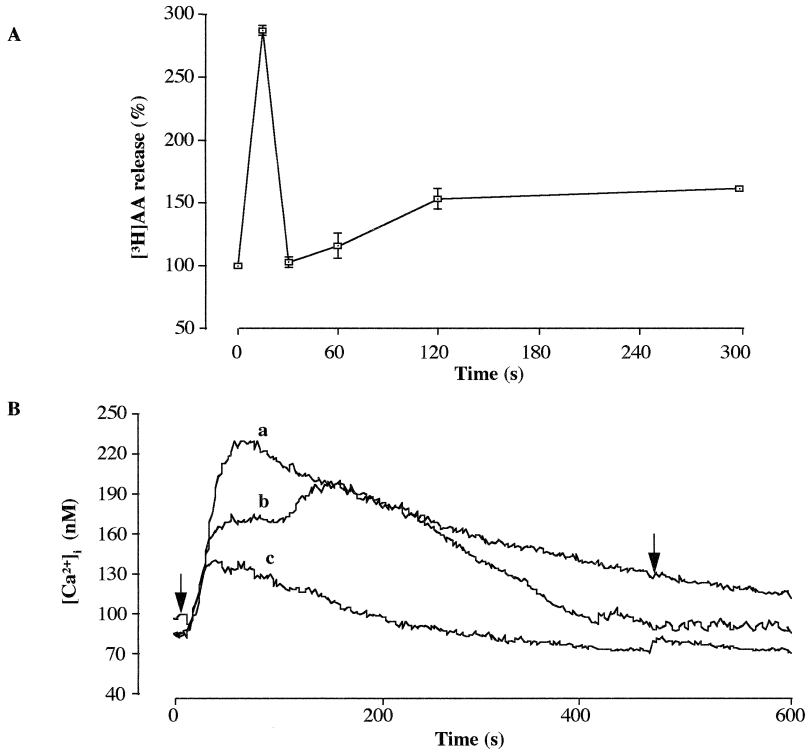

Fig. 3. Involvement of arachidonic acid in the histamine-induced intracellular Ca^{2+} increase. (A) [^{3}H]arachidonic acid-labelled WI26VA4 cells were stimulated with histamine (10^{-4} M), and the reaction was stopped at various times between 0 and 300 s. The amount of [^{3}H]arachidonic acid released was expressed as percentage of control. The data are the means ± S.E.M. of three experiments. (B) Fluo 3-loaded WI26VA4 cells were stimulated (the first arrow) with arachidonic acid at various concentrations (5×10^{-5} M, a; 5×10^{-6} M, b; 10^{-6} M, c). Each arachidonic acid stimulation was followed by histamine stimulation (the second arrow). All traces are representative of at least three separate experiments.

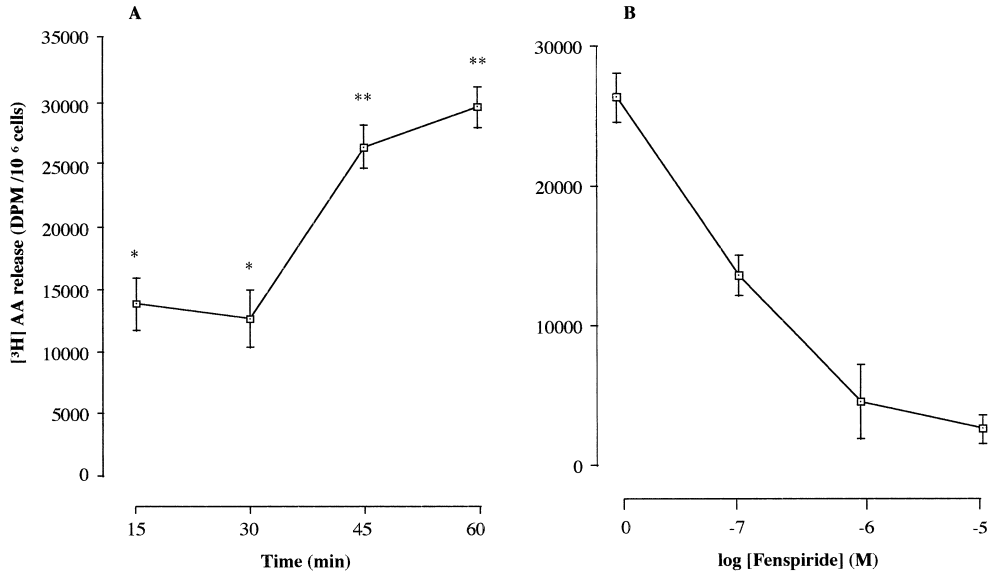

Fig. 4. Interference of fenspiride with histamine-induced [^{3}H]arachidonic acid release. (A) [^{3}H]arachidonic acid-labelled WI26VA4 cells were stimulated with histamine (10^{-4} M) and the reaction was stopped at various times between 15 and 60 min. The amount of [^{3}H]arachidonic acid released was expressed as the difference between histamine and control. Results are presented as the means ± S.E.M. of three experiments. Asterisks indicate a significant increase ($* P < 0.05$; $** P < 0.01$). (B) [^{3}H]arachidonic acid-labelled WI26VA4 cells were stimulated with histamine (10^{-4} M) without or after preincubation with fenspiride at various concentrations. The reaction was stopped after 45 min. The amount of [^{3}H]arachidonic acid released was expressed as the difference between histamine plus fenspiride and fenspiride alone. Results are presented as the means ± S.E.M. of three experiments.

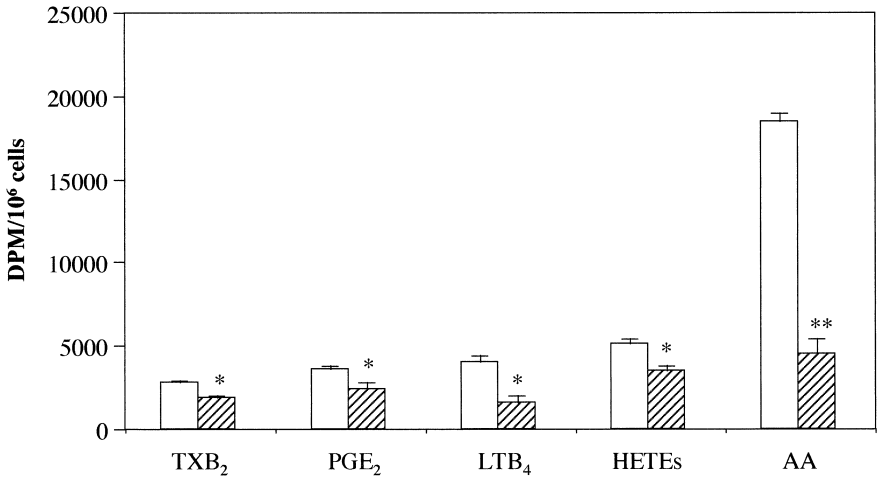

Fig. 5. Interference of fenspiride with eicosanoid production. [^{3}H]arachidonic acid-labelled WI26VA4 cells were stimulated with histamine (10^{-4} M) without (open bars) or after preincubation with fenspiride (10^{-5} M, diagonal lined bars). The reaction was stopped after 45 min. Eicosanoid production was analysed by T.L.C. Results are presented as the means ± S.E.M. of three experiments. Asterisks indicate a significant reduction of eicosanoid production ($* P < 0.05$; $** P < 0.01$).

M, 5×10^{-5} M) elicited a concentration-dependent increase in intracellular Ca^{2+} (Fig. 3B). Histamine failed to induce Ca^{2+} changes when the cells had been first stimulated by arachidonic acid. All these results suggest that a histamine-induced short-term arachidonic acid release acts as a second messenger inducing an intracellular Ca^{2+} increase.

We then investigated whether the inhibition of the histamine-induced intracellular Ca^{2+} increase by fenspiride resulted from the inhibition of phospholipase A_2 activity or from interference with the effect of arachidonic acid on Ca^{2+} changes. To answer this question, we analysed the histamine-induced arachidonic acid release at 15 s in the presence of 10^{-5} M fenspiride, the dose which most inhibited the histamine-induced intracellular Ca^{2+} increase. Fenspiride significantly abolished the phospholipase A_2-induced arachidonic acid release (91 ± 8% of inhibition, $n = 3$). In contrast, fenspiride failed to inhibit the arachidonic acid-induced Ca^{2+} increase (140 ± 10 nM with fenspiride vs. 145 ± 8 nM without fenspiride, $n = 6$).

3.4. Release of arachidonic acid and eicosanoids in response to histamine

The second phase of [^{3}H]arachidonic acid release occurred between 30 and 60 min (Fig. 4A). Analysis of the second increase indicated that, in contrast to the first phase, arachidonic acid and eicosanoids were present. At 45 min, when [^{3}H]arachidonic acid release was significant ($P < 0.01$ vs. control), histamine significantly induced the production of arachidonic acid metabolites (Fig. 5). For the cyclooxygenase metabolites, prostaglandin E_2 and thromboxane B_2 production was significant ($P < 0.05$ vs. control). Among the lipoxygenase metabolites, WI26VA4 cells significantly released leukotriene B_4 and hydroxyeicosatetraenoic acid ($P < 0.05$ vs. control). However, in response to histamine stimulation, WI26VA4 cells released small amounts of arachidonic acid metabolites compared to the amount of free arachidonic acid. Histamine-stimulated phospholipase A_2 activity seems to be more important than histamine-stimulated cyclooxygenase and lipoxygenase activities in this human lung epithelial cell line, according to the results obtained with the human bronchial epithelial BEAS-2B cell line (Noah et al., 1991). We observed a dose-dependent decrease of arachidonic acid and eicosanoid production in the presence of fenspiride (Fig. 4B). The formation of both cyclooxygenase and lipoxygenase products was also inhibited by fenspiride (Fig. 5).

4. Discussion

In this study, fenspiride inhibited two histamine responses in the human lung epithelial WI26VA4 cell line. First, fenspiride abolished the histamine-induced intracellular Ca^{2+} increase measured in Fluo-3-loaded WI26VA4 cells. Secondly, fenspiride blocked the histamine-induced release of arachidonic acid and eicosanoid, evaluated in [^{3}H]arachidonic acid-labelled cells. Moreover, we showed that the cellular mechanism of the effects of fenspiride seems to be explainable by the inhibition of arachidonic acid formation and subsequent changes in intracellular Ca^{2+} concentration.

Epithelium represents more than a passive barrier for the separation of internal and external milieu in the lung, since epithelial cells are implicated in the response to injury, according to the earliest descriptions of the cellular components of airway inflammation (Levine et al., 1995). An interaction between epithelial cells and mast cells is often suggested. Barnes and Liew (1995) proposed that airway epithelial cells may sensitize mast cells by amplify-

ing and perpetuating the subset 2 of helper T (Th2) cell-mediated inflammatory response. Furthermore, several studies have shown that histamine produced by mast cells may activate epithelial cells to produce inflammatory mediators such as cytokines or eicosanoids (Takizawa et al., 1995; Harris and Hanrahan, 1993; Noah et al., 1991).

Among the eicosanoids produced by airway epithelial cells, 15-hydroxyeicosatetraenoic acid plays an important role in the recruitment of macrophages and neutrophils (Levine et al., 1995). Although our separation method for arachidonic acid metabolites could not separate 5- and 15-hydroxyeicosatetraenoic acids, we measured a significant increase in their release. A high level of free arachidonic acid was also released by WI26VA4 cells. Several studies have shown how macrophages and neutrophils use hydroxyeicosatetraenoic acids and free arachidonic acid released by epithelial cells, to produce new amounts of eicosanoids (Zhou et al., 1995; Peters-Golden and Feyssa, 1993; Chavis et al., 1992).

In models of airway inflammation, a response to histamine was seen after epithelial cell injury due to tumor necrosis factor-α (TNF-α) and interleukine-1β released by macrophages (Levine et al., 1995; Barnes and Liew, 1995). It has been shown that TNF-α and interleukine-1β can allow priming of arachidonic acid metabolism, inducing phospholipase A_2 and cyclooxygenase-2 gene and protein expression in human epithelial cells. The capacity of these cells to produce eicosanoids in response to various stimuli can therefore be enhanced by this priming (Croxtall et al., 1996; Wu et al., 1996; Mitchell et al., 1994). The small quantities of eicosanoid production that were measured may be explained by the absence of priming of cells used in our study. Indeed, histamine stimulation of WI26VA4 cells after TNF-α and interleukine-1β action could have a more marked effect on eicosanoid production.

Considering all the above data on the cooperation of inflammatory cells (macrophages, neutrophils, mast cells) and epithelial cells leading to eicosanoid production, the present demonstration that fenspiride inhibits the histamine-induced [^{3}H]arachidonic acid release confirms the anti-inflammatory properties of this molecule.

The important role of Ca^{2+} in histamine signal transduction has been demonstrated in distinct airway epithelial cell lines (Harris and Hanrahan, 1993; Noah et al., 1991). We showed that the histamine H_1 receptor induces an intracellular Ca^{2+} increase using phospholipase A_2 pathway in the human lung epithelial WI26VA4 cell line. Van der Zee et al. (1995) have shown that arachidonic acid, released after histamine H_1 receptor stimulation in DDT_1 MF-2 cells, functions as a second messenger to activate plasma membrane Ca^{2+} channels, promoting Ca^{2+} entry from the extracellular space, but after phospholipase C-dependent release of Ca^{2+} from the intracellular Ca^{2+} store. In our study, neomycine and U-73122, two phospholipase C inhibitors, failed to inhibit the histamine-induced intracellular calcium increase. However, as these compounds, applied extracellularly, are not considered as specific inhibitors of inositide breakdown (Alter et al., 1994; Sipma et al., 1996), we cannot exclude the involvement of inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) in the histamine Ca^{2+} response.

In addition, we now showed that the fenspiride inhibition of the histamine H_1 receptor-induced intracellular Ca^{2+} concentration increase can be explained by inactivation of phospholipase A_2. Indeed, the histamine-induced short-term arachidonic acid peak disappeared in the presence of fenspiride. Furthermore, it has been shown, using other cells, that fenspiride may interfere with histamine-activated phospholipase C and $Ins(1,4,5)P_3$ accumulation (Evrard et al., 1991). These results did not allow us to clearly specify the target of fenspiride action, that is either directly histamine H_1 receptor activation, or signalling pathways coupled to the histamine H_1 receptor. Recently, Morcillo et al. (ATS international conference, San Francisco, 1997) suggested that fenspiride was an effective inhibitor of type IV phosphodiesterases that hydrolyze cyclic AMP, and that this action could contribute to its airway effects. Knowing that cyclic AMP inhibits histamine H_1 receptor effects (Arrang et al., 1995; Leurs et al., 1995), we plan to investigate whether or not fenspiride may act through a cyclic AMP-dependent protein kinase and the desensitization of the histamine H_1 receptor.

5. Conclusion

This study confirmed the anti-inflammatory properties of fenspiride. Particularly, fenspiride interfered with the histamine H_1 receptor-induced intracellular Ca^{2+} increase and eicosanoid production. The effect of fenspiride on histamine-induced intracellular Ca^{2+} rise may be explained by the phospholipase A_2 inactivation in the human lung epithelial WI26VA4 cells.

Acknowledgements

We thank Marie-Hélène Séguélas for her expert technical assistance with cell culture and the determination of arachidonic acid and eicosanoid production, and Alex Wong for language correction of the manuscript. This study was supported in part by the Laboratoires Servier and Fabio Quartulli is the recipient of a grant from Laboratoires Servier.

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