Histamine activates an intracellular Ca²⁺ signal in normal human lung fibroblast WI-38 cells

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Histamine is an inflammatory mediator that can be released from mast cells to induce airway remodeling and cause persistent airflow limitation in asthma. In addition to stimulating airway smooth muscle cell constriction and hyperplasia, histamine promotes pulmonary remodeling by inducing fibroblast proliferation, contraction, and migration. It has long been known that histamine receptor 1 (H1R) mediates the effects of histamine on human pulmonary fibroblasts through an increase in intracellular Ca²⁺ concentration ([Ca²⁺]ᵢ), but the underlying signaling mechanisms are still unknown. Herein, we exploited single-cell Ca²⁺ imaging to assess the signal transduction pathways whereby histamine generates intracellular Ca²⁺ signals in the human fetal lung fibroblast cell line, WI-38. WI-38 fibroblasts were loaded with the Ca²⁺-sensitive fluorophore, FURA-2/AM, and challenged with histamine in the absence and presence of specific pharmacological inhibitors to dissect the Ca²⁺ release/entry pathways responsible for the onset of the Ca²⁺ response. Histamine elicited complex intracellular Ca²⁺ signatures in WI-38 fibroblasts throughout a concentration range spanning between 1 µM and 1 mM. In accord, the Ca²⁺ response to histamine adopted four main temporal patterns, which were, respectively, termed peak, peak-oscillations, peak-plateau-oscillations, and peak-plateau. Histamine-evoked intracellular Ca²⁺ signals were abolished by pyrilamine, which selectively blocks H1R, and significantly reduced by ranitidine, which selectively inhibits H2R. Conversely, the pharmacological blockade of H3R and H4R did not affect the complex increase in [Ca²⁺]ᵢ, evoked by histamine in WI-38 fibroblasts. In agreement with these findings, histamine-induced intracellular Ca²⁺ signals were initiated by intracellular Ca²⁺ release from the endoplasmic reticulum through inositol-1,4,5-trisphosphate (InsP₃) receptors (InsP₃R) and sustained by store-operated Ca²⁺ channels (SOCs). Conversely,
Introduction

Asthma is a heterogeneous disease, generally characterized by chronic inflammation of the airways, defined by a clinical history of respiratory symptoms, such as wheezing, shortness of breath, chest tightness, and cough that vary in intensity and frequency, along with variable expiratory airflow (GBD Diseases and Injuries Collaborators, 2020). About 300 million people suffer from asthma worldwide and it is likely that, by 2025, another 100 million people will be affected by this disease (Dharmage et al., 2019).

The complex network of inflammatory responses in the pathophysiology of asthma involves the release of inflammatory mediators, such as cytokines, chemokines, proteases, and histamine (Murdoch and Lloyd, 2010). Histamine has been a widely recognized inflammatory mediator released from mast cells and could play a key role in the pathophysiology of asthma (Yamauchi and Ogasawara, 2019). Tomioka et al. (1984) estimated that the number of mast cells and the concentration of histamine in bronchoalveolar lavage fluid of asthmatic patients was higher than that of healthy subjects. In addition, Carroll et al. (2002) demonstrated that mast cell degranulation is related to the severity of asthma. Salomonsson et al. (2019) recently reported that elevated levels of circulating mast cell progenitors are related to reduced lung function in asthmatic patients.

In vitro studies demonstrated that histamine stimulated lung fibroblast collagen synthesis (Garbuzenko et al., 2004; Veerappan et al., 2013), migration (Kohyama et al., 2010), proliferation (Jordana et al., 1988; Veerappan et al., 2013) and human lung myofibroblast contraction (Horie et al., 2014). However, the transduction mechanisms whereby histamine leads to these effects in lung fibroblasts are still unclear. Histamine is thought to act by stimulating one or more of four types of plasmalemmal histaminergic receptor (HR) (H1R, H2R, H3R, and H4R) (Panula et al., 2013; Panula, 2021). The expression of H1R and, in less proportion, H2R has been reported in normal human lung fibroblasts (Veerappan et al., 2013) and their participation in promoting lung fibroblast activation has been proposed (Jordana et al., 1988; Garbuzenko et al., 2004; Kunzmann et al., 2007; Veerappan et al., 2013). In addition, H4R could promote lung fibroblast migration (Kohyama et al., 2010). Stimulation of H1R results in an increase in intracellular Ca2+ concentration ([Ca2+]i) in human valvular myofibroblasts (Liang et al., 2003), pterygial derived fibroblasts (Maini et al., 2002), human gingival fibroblasts (Niisato et al., 1996; Ogata et al., 1999; Gutiérrez-Venegas and Rodríguez-Pérez, 2012), rheumatoid synovial fibroblasts (Zenmyo et al., 1995) human skin fibroblasts (Johnson et al., 1990), human subcutaneous fibroblasts (Pinheiro et al., 2013), and human lung fibroblasts (Zheng et al., 1994; Horie et al., 2014). While H3R are predominantly located in neurons (Abdulrazzaq et al., 2022), H4R are preferentially expressed in cells of the immune system and in blood forming organs, especially in mast cells, dendritic cells, basophils, eosinophils, monocytes, and T lymphocytes (Sarasola et al., 2021). However, immunostaining demonstrated that also human dermal fibroblasts express the H4R (Ikawa et al., 2008). Signaling mechanisms for the H4R receptor are much less well understood but appear to involve an increase in [Ca2+]i (Panula et al., 2015).

An elevation in [Ca2+]i in fibroblasts is key to protein synthesis, transcription factor activation, migration, progression through the cell cycle, and cell viability (Janssen et al., 2015). Resting [Ca2+]i is maintained at very low levels (~100–200 nM), while the extracellular Ca2+ concentration is 1000-fold higher (> 1 mM) and the Ca2+ concentration in the primary intracellular Ca2+ store, the endoplasmic reticulum (ER), fluctuates between 100–800 µM (Sadras et al., 2021a). An array of agonists, such as bradykinin, thrombin, trypsin, adenosine triphosphate, angiotensin II, and histamine, increases the [Ca2+]i, in pulmonary fibroblasts at concentrations ranging from approximately 100 nM to 0.1 mM (Janssen et al., 2015). The [Ca2+]i can be increased by two main mechanisms upon cellular stimulation: the first one is through the release of Ca2+ from the ER and the second one is through the inflow of Ca2+ from the extracellular medium. In several cell types, histamine bind to the Gq-protein-coupled receptor (GqPCR), H1R, which activates phospholipase C (PLC). PLC hydrolyzes a membrane phospholipid, phosphatidylinositol 4, 5 bisphosphate (PIP2), to produce inositol-1, 4, 5-trisphosphate (InsP3). InsP3 triggers Ca2+ mobilization from the ER through InsP3 receptors (InsP3R) that
are located on the ER membrane and can in turn activate adjacent ryanodine receptors (RyR) through the process of Ca2+-induced Ca2+ release (CICR) (Paltauf-Doburzynska et al., 2000; Clapham, 2007; Horie et al., 2014; Ishida et al., 2014; Berra-Romani et al., 2020). Extracellular Ca2+ can permeate the plasma membrane through a wide variety of ion channels, including voltage-operated channels (VOC) (Janssen et al., 2015; Rahman et al., 2016) and agonist-operated channels, which comprise three types of channels: 1) receptor-operated channels (ROC) (Saliba et al., 2015), which are ionotropic receptors stimulated by direct ligand binding, 2) second messenger-operated channels (SMOC), which are activated by intracellularly generated mediators, such as cyclic nucleotides, diacylglycerol (Hofmann et al., 2017), and arachidonic acid, and 3) store-operated Ca2+ channels (SOC), which are the main Ca2+ entry pathway in non-excitable cells. In the ER, Stromal Interaction Molecules (STIM1/2) act as sensors of ER Ca2+ concentration that, after a reduction in intraluminal Ca2+, multimerize and translocate towards peripheral ER cisternae to functionally interact with the Ca2+-permeable Orai channels on the plasma membrane (Bendiks et al., 2020). The following influx of Ca2+ has been termed store-operated Ca2+ entry (SOCE) and mediates agonist-induced Ca2+ influx in human fibroblasts isolated from several tissues, including lungs (Guzmán-Silva et al., 2015; Vazquez-de-Lara et al., 2018).

It has been demonstrated that histamine triggers an increase in [Ca2+], also in human lung fibroblasts (Zheng et al., 1994; Horie et al., 2014). However, the molecular mechanisms implicated in this response, which are likely to regulate the multiple fibroblast function involved in asthma remodeling, remain to be elucidated. Therefore, this study aimed to examine for the first time the mechanisms underlying histamine-induced increase in [Ca2+], in fetal human pulmonary WI-38 fibroblasts.

Materials and methods

Cell culture

Human fetal lung fibroblast cell lines were purchased from American Type Culture Collection, Collection WI-38 (ATCC® CCL-75™) and cultured to 75% confluence in DMEM (Dulbecco’s Modified Eagle Medium) culture medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in an atmosphere of 95% O2 and 5% CO2. Fibroblasts from passages 5–10 were seeded on coverslips for 24 h and then incubated for 48 h in medium devoid of serum.

Physiological solutions

Physiological saline solution (PSS) with the following composition (in mM) 150 NaCl, 6 KCL, 1.5 CaCl2 1 MgCl2, 10 glucose, 10 HEPES was used for this study. To obtain Ca2+-free physiological saline solution (0Ca2+), Ca2+ was replaced with 2 mM NaCl and 0.5 mM EGTA was added as a Ca2+ chelator. Osmolarity was measured with an osmometer (Wescor 5500, Logan, UT, United States) solutions were adjusted to pH 7.4 with NaOH.

Measurement of [Ca2+]i

The technique for Ca2+ measurement in fibroblasts has been previously described (Guzmán-Silva et al., 2015), and is explained in detail below. Fibroblasts attached to coverslips were washed twice PSS and incubated with 3 μM FURA-2 acetoxymethyl ester (FURA-2-AM) in PSS for 30 min at room temperature (21°C–23°C). Cells were incubated for 30 min in PSS free of FURA-2-AM. The coverslips were washed and fixed to the bottom of a Petri dish using a drop of silicone. The Petri dish was mounted on a stage of the Axioskop upright epifluorescence microscope (Carl Zeiss, Oberkochen, Germany), equipped with a 100 W mercury lamp (OSRAM HBO 50). A Zeiss X63 Achroplan objective (water immersion, working distance 2.0 mm, numerical aperture 0.9) was used to visualize fibroblasts. Cells were alternately excited at 340 nm and 380 nm using a filter spinning wheel with a shutter (Lambda 10, Sutter Instrument, Novato, CA, United States) and light emitted was detected at 510 nm. The Ca2+ signal was measured in individual fibroblasts (Zheng et al., 1994; Remigante et al., 2021). Experiments were performed at room temperature (21°C–23°C). All experiments were performed in triplicate using 3 different passages of fibroblasts for each of the conditions.

RT-qPCR

Total RNA was isolated from fibroblasts using QIAzol Lysis Reagent (Qiagen SpA, Milan, Italy), and reverse transcription was performed as described in (Ferrera et al., 2021; Negri et al., 2021). Reverse transcription was always performed in the presence (positive) or in the absence (negative control) of the reverse transcriptase enzyme (not shown), as shown elsewhere...
(Zuccolo et al., 2019; Zuccolini et al., 2022). cDNA amplification was performed using KAPA SYBR FAST qPCR Master Mix (KAPA BIOSYSTEMS, United States), and the primers used for amplification are listed in Table 1. The conditions were as follows: initial denaturation at 95 °C for 5 min; 40 cycles of denaturation at 95°C for 10 s; annealing and extension at 60°C for 30 s, PCR products were separated on a 3% Nusieve® (2:1) gel agarose, stained with ethidium bromide, and acquired with the iBrightTM CL1000 Imaging System (Thermo Fisher Scientific Inc., United States). The molecular weight of the PCR products was compared with the DNA molecular weight marker VIII (Roche Molecular Biochemicals, Italy).

### Data analysis

For the acquisition of fluorescence values, ImageJ software was used, and Origin Pro 2021 and GraphPad Prism 8.0 were used for graphing and statistical analysis of the results.

### Statistical design

Data were expressed as mean ± standard error (SE). Non-Gaussian data, identified by the D’Agostino and Pearson omnibus normality test (p ≤ 0.05) were statistically analyzed by the nonparametric Mann-Whitney test.

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**Table 1** Primer sequences used for reverse transcription/polymerase chain reaction.

| Gene   | Primer sequences                          | Size (bp) | Accession number |
|--------|------------------------------------------|-----------|------------------|
| Orai1  | Forward-5'-AGTTACTCGAGGTGATGAG-3'       | 257       | NM_032790.3      |
|        | Reverse-5'-ATGCAGGTGCTGATCATGAG-3'      |           |                  |
| Orai2  | Forward-5'-CCATAAGGGCATGAGTACC-3'       | 334       | NM_00126340.1    |
|        | Reverse-5'-CAGTTGTTGAGTTGGCTCA-3'       |           | NM_032831.2      |
| Orai3  | Forward-5'-CCAAGCTAAGCTCAGGCC-3'        | 159       | NM_152,288.2     |
|        | Reverse-5'-CAAAGAGTGGCAAGCCACACA-3'     |           |                  |
| Stim1  | Forward-5'-CCTCAGTATGGAGAGACCTT-3'      | 347       | NM_003156.3      |
|        | Reverse-5'-TCCTGAAAGTTCAGCTGACACT-3'    |           |                  |
| Stim2  | Forward-5'-AAACACAGCCATCTGACAG-3'       | 186       | NM_020860.2      |
|        | Reverse-5'-GGGAAGTTGTTGTCCTTTGA-3'      |           |                  |
| InsP3R1| Forward-5'-CAAAACACTGACCACAGCT-3'       | 180       | ENSG00000150995  |
|        | Reverse-5'-CTCTCATGGGATCTTCTCCT-3'      |           |                  |
| InsP3R2| Forward-5'-ACCTTGGCCATGAGTGA-3'         | 158       | ENSG00000123104  |
|        | Reverse-5'-CCITTTGTTCTGCTTTGGC-3'       |           |                  |
| InsP3R3| Forward-5'-TGCTCTATGACACCTTGG-3'        | 173       | ENSG00000096433  |
|        | Reverse-5'-TGCTCTGCTTATTGCTGCTT-3'      |           |                  |
| RyR1   | Hs00166991 Thermo Fisher Scientific      | 75        | NM_000540.3      |
| RyR2   | Hs00181461 Thermo Fisher Scientific      | 65        | NM_001035        |
| RyR3   | Hs00168821 Thermo Fisher Scientific      | 63        | NM_001036.6      |
| Trpc1  | Forward-5'-ATCCTACATGCTGGTGCAGAA-3'     | 307       | NM_00130698.1    |
|        | Reverse-5'-AAACAGCAAGCAGGTTGCCC-3'      |           | v                 |
| Trpc3  | Forward-5'-GGAGATCTGGAATCAGGACAGA-3'    | 336       | NM_00130698.1    |
|        | Reverse-5'-AAGACCACCAGGAAGATGAGA-3'     |           | NM_003305.2      |
| Trpc4  | Forward-5'-ACCTGGGACCTCTGCAAAATA-3'     | 300       | NM_06179.2       |
|        | Reverse-5'-ACATGGTGCCACACAAAC-3'        |           | va                |
| Trpc5  | Forward-5'-AGATGACACAGCAGTAGGAGAAG-3'   | 221       | NM_00135955.1    |
|        | Reverse-5'-AGACACGAGCAGGGAAACAGGA-3'    |           | NM_00135956.1    |
| Trpc6  | Forward-5'-AGCTTGTCCAGGCCCATAAA-3'      | 341       | NM_004625.1      |
|        | Reverse-5'-AAGAGTTCTCATAGGGAGGAC-3'     |           |                  |
| Trpc7  | Forward-5'-CAGTTGCAAACCTGCTAGA-3'       | 387       | NM_020389.1      |
|        | Reverse-5'-CATTCCACATCATGAAGGCCA-3'     |           |                  |
Whitney test for two groups and Kruskal–Wallis for more than two groups. For normal data, an unpaired Student t-test for two groups and ANOVA for more than two groups were used. A value of $p \leq 0.05$ was considered statistically significant.

Histamine concentration-response data were adjusted by the following Eq. 1:

$$Y = \frac{100}{1 + \frac{[\text{Histamine}]}{EC_{50}}}$$  \hspace{1cm} (1)

where $Y$ is the response (relative to the $\text{Ca}^{2+}$ transient amplitude), [Histamine] is the histamine concentration and the mean maximal effective concentration ($EC_{50}$) is the [Histamine] that induced 50% of the maximal response.

**Results**

**Histamine causes a heterogeneous $\text{Ca}^{2+}$ signal in human lung fibroblasts of the WI-38 cell line**

Using digital fluorescence imaging with FURA-2/AM $[\text{Ca}^{2+}]$, was measured simultaneously in several individual fibroblasts from the same population. A 22.5% of WI-38 fibroblasts displayed spontaneous $\text{Ca}^{2+}$ oscillations, as also reported in human cardiac fibroblasts (Chen et al., 2010). These cells were, therefore, discarded from subsequent analysis (Faris et al., 2022). Application of histamine (300 μM), even in cells in the same microscopic field, elicited $\text{Ca}^{2+}$ signals showing heterogeneous kinetics, which were classified into 4 different patterns: the first consisted of a rapid and transient increase in $[\text{Ca}^{2+}]$, termed peak (59/306 cells, 19.28%, Figure 1A); the second in a peak followed by cyclic increases and decreases in $[\text{Ca}^{2+}]$, termed peak-oscillations (198/306 cells, 64.70%, Figure 1B); the third in a peak with oscillations and a sustained increase in $[\text{Ca}^{2+}]$, termed peak-plateau-oscillations (41/306 cells, 13.39%, Figure 1C); and finally, the fourth pattern which was the least frequent, consisted of a peak accompanied by a plateau, and was termed peak-plateau (2/306 cells, 0.65%, Figure 1D). Only 1.96% (6/306 cells) of the analyzed fibroblasts did not respond to histamine 300 μM.

**Histamine generates a $\text{Ca}^{2+}$ signal in a concentration-dependent manner in WI-38 human lung fibroblasts**

Typical recordings of the $\text{Ca}^{2+}$ signals evoked by different histamine concentrations (100 nM–1 mM) are shown in Figure 2A. Histamine did not elicit any discernible increase in
[Ca\textsuperscript{2+}], at very low concentrations, such as 100 and 300 nM. The Ca\textsuperscript{2+} response to histamine appeared at 1–3 µM; at these concentrations, the Ca\textsuperscript{2+} signal arising in most WI-38 cells displayed a single peak in response to agonist stimulation. However, at 10 µM histamine, the Ca\textsuperscript{2+} peak was followed by a short train of consecutive Ca\textsuperscript{2+} oscillations. The number of oscillations over 60 min of histamine application was increased at histamine concentrations ranging from 30 µM up to 1 mM. The non-cumulative concentration-response curve of histamine-induced elevation in [Ca\textsuperscript{2+}], which was calculated by fitting the data to Eq. 1, as shown in Materials and methods, which yielded EC\textsubscript{50} values of 4.96 and 5.25 µM for peak amplitude (black line) and plateau amplitude (blue line), respectively. Data points were obtained of at least 19 cells. (C) Percentage of cells that presented each of the Ca\textsuperscript{2+} response patterns indicated in function of histamine concentrations applicated to WI-38 fibroblasts. (D) Data points are the mean ± SE of the number of oscillations measured over the first 400 s after histamine application, plotted against the logarithm of histamine concentration. The continuous curve was obtained by fitting the data to Eq. 1, as shown in Materials and methods, which yielded EC\textsubscript{50} values of 2.38 µM.

Desensitization of the Ca\textsuperscript{2+} response by repeated stimulation of WI-38 human lung fibroblasts with histamine

Homologous desensitization is a feature of GqPCRs, including H1R (Chen et al., 2014; Burghi et al., 2021).
Figure 3A shows a typical Ca²⁺ recording from a WI-38 fibroblast exposed to three consecutive applications of 300 µM histamine followed by PSS washout. Histamine elicited a similar Ca²⁺ response consisting in an initial Ca²⁺ peak followed by sustained plateau, superimposed by Ca²⁺ oscillations. However, the peak and plateau amplitudes (Figure 3B), as well as the number of oscillations (Figure 3C), were significantly reduced by repetitive histamine stimulation. These data suggest that the application of a maximal concentration of histamine (300 µM) to the same fibroblast leads to receptor desensitization.

Histamine-evoked elevation in [Ca²⁺]i in WI-38 human lung fibroblasts is primarily mediated through activation of H1R and to a lesser extent through H2R

In order to elucidate the HR subtype through which histamine triggers an intracellular Ca²⁺ signal in WI-38 human lung fibroblasts, specific HR antagonists were used: for H1Rs pyrilamine (100 µM), for H2Rs ranitidine (50 µM), for H3R clobenpropit (50 µM) and, finally, for the H4R receptor, NJ7777120 (10 µM). After 30 min preincubation and in the continuous presence of the histaminergic antagonists, histamine (300 µM) was applied as indicated by the green bars. In order to confirm cell viability, arachidonic acid (AA) 50 µM was applied in cells in which histamine failed to induce an increase in [Ca²⁺]i (Berra-Romani et al., 2019). Pharmacological manipulation of HR in WI-38 human lung fibroblasts revealed that H1R blockage completely abolished the histamine-evoked Ca²⁺ signal (Figure 4A), H2R blockage significantly decreased the amplitude of the Ca²⁺ signal (Figure 4B), while H3R (Figure 4C) and H4R blockage (Figure 4D) had no significant effect. In Figure 4E, the statistical comparison between the mean ± SE peak amplitude of the Ca²⁺ signals evoked by histamine 300 µM in the absence (Ctrl) and presence of the different antihistaminergic receptors antagonists (H1R, H2R, H3R, and H4R) are summarized. These results indicate that, for the [Ca²⁺]i elevation to take place in the lung fibroblast cell line, WI-38, the activation of mainly H1R and, to a lesser extent, H2R is necessary, whereas H3R and H4R seem to play no role.
Histamine-evoked \( \text{Ca}^{2+} \) signals in WI-38 lung fibroblasts do not involve \( \text{G}_{\alpha_{i/o}} \) activation but require \( \text{PLC}\beta \) recruitment and \( \text{ER Ca}^{2+} \) release

The results shown in Figures 4C–E suggest that H3R and H4R do not participate in histamine-activated \( \text{Ca}^{2+} \) signaling. H3R and H4R are canonically coupled to \( \text{G}_{\alpha_{i/o}} \) proteins (Haas et al., 2008). Therefore, a reliable strategy to corroborate the lack of H3R and H4R involvement in histamine response would be to demonstrate that pertussis toxin (PT), a selective inhibitor of \( \text{G}_{\alpha_{i/o}} \) protein signalling, does not modify histamine-evoked \( \text{Ca}^{2+} \) signals. Indeed, as shown in Figure 5A, preincubation (30 min) with 100 ng/ml PT did not prevent histamine from increasing intracellular \( \text{[Ca}^{2+}]_i \) in WI-38 fibroblasts. In accord, the amplitude of initial \( \text{Ca}^{2+} \) transient (peak) was not statistically different from untreated cells (Figure 5B). These results confirm that histamine-evoked increase in \( \text{[Ca}^{2+}]_i \) in human lung WI-38 fibroblasts is insensitive to the inhibition of \( \text{G}_{\alpha_{i/o}} \) by PT (Figures 5A,B). After confirming that H1R and H2R are involved in the \( \text{Ca}^{2+} \) response to histamine, we turned to dissect out the molecular

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**FIGURE 4**

Dissection of histaminergic receptors (HR) responsible for histamine-evoked \( \text{Ca}^{2+} \) signal in WI-38 human lung fibroblasts. (A) Typical recording of histamine-evoked \( \text{Ca}^{2+} \) signal in the presence of the H1R antagonist, pyrilamine (100 \( \mu \text{M} \)). (B) Typical recording of the histamine-evoked \( \text{Ca}^{2+} \) signal in the presence of the H2R antagonist, ranitidine (50 \( \mu \text{M} \)). (C) Typical recording of the histamine-evoked \( \text{Ca}^{2+} \) signal in the presence of the H3R antagonist, clobenpropit (50 \( \mu \text{M} \)). (D) Typical recording of histamine-evoked \( \text{Ca}^{2+} \) signal in the presence of H4R antagonist, JNJ-7777120 (10 \( \mu \text{M} \)). All histaminergic antagonists were preincubated for 30 min prior to histamine application (incubation time not shown). For experiments showed in (A,C), arachidonic acid (AA 50 \( \mu \text{M} \)) was applied after histamine application to corroborate cell viability. (E) Mean ± SE of the peak amplitude of the \( \text{Ca}^{2+} \) transient evoked by histamine (300 \( \mu \text{M} \)) in the absence (Ctrl) and presence of the HR antagonists: pyrilamine 100 \( \mu \text{M} \), ranitidine 50 \( \mu \text{M} \), clobenpropit 50 \( \mu \text{M} \), and JNJ-7777120 (10 \( \mu \text{M} \)). The numbers in the figure represents the number of cells studied. Comparison between groups was performed using the Kruskal–Wallis test (* = \( p \leq 0.05 \)) (ns = no statistically relevant differences between groups).
underpinnings of the Ca\textsuperscript{2+} transient by using the following drugs: 1) U73122 (10 μM), a selective inhibitor of PLC (Moccia et al., 2006; Berra-Romani et al., 2012; Berra-Romani et al., 2020); 2) U73343 (10 μM), an inactive analogue of U73122 (Guzmán-Silva et al., 2015); and 3) cyclopiazonic acid (CPA) (10 μM), a selective inhibitor of sarco-endoplasmic reticulum calcium ATPase (SERCA) pump (Guzmán-Silva et al., 2015; Berra-Romani et al., 2020). The results obtained showed that PLC inhibition upon preincubation (15 min) with U73122 suppresses histamine-evoked Ca\textsuperscript{2+} signal (Figures 5C,F), whereas its inactive analog, U73343, does not affect the Ca\textsuperscript{2+} response (Figures 5D,F). Likewise, ER emptying via Ca\textsuperscript{2+} leak channels after SERCA inhibition with CPA in an extracellular Ca\textsuperscript{2+}-free environment (0Ca\textsuperscript{2+}) prevented the Ca\textsuperscript{2+} response to histamine (Figures 5E,F). These results indicate that the Ca\textsuperscript{2+} transient generated

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**FIGURE 5**

Histamine-evoked Ca\textsuperscript{2+} signals in WI-38 human lung fibroblasts do not involve G\textsubscript{i/o} activation but require PLC and ER Ca\textsuperscript{2+} release. (A) Typical recording of the Ca\textsuperscript{2+} response to histamine (300 μM) in fibroblasts preincubated for 30 min with 100 ng/ml pertussis toxin (PT). (B) Mean ± SE of the peak amplitude of histamine-evoked Ca\textsuperscript{2+} transients in the absence (green bar) and presence of pertussis toxin (gray bar). Comparison between groups was performed using the Student’s t-test (ns = no statistically relevant differences between groups). (C) Typical recording of the effect of histamine (300 μM) on Ca\textsuperscript{2+} signal in cells pretreated for 15 min with U73122 (10 μM), a specific PLC inhibitor. (D) Typical recording of the Ca\textsuperscript{2+} response to histamine (300 μM) in cells pretreated for 15 min with U73343 (10 μM), an inactive analog of U73122. (E) Representative recording of the Ca\textsuperscript{2+} signal evoked by histamine (300 μM) in cells pretreated with CPA (10 μM) in the absence of extracellular Ca\textsuperscript{2+} (0Ca\textsuperscript{2+}). (F) Mean ± SE of the peak Ca\textsuperscript{2+} response to histamine (300 μM; green bar) in the presence of U73122 10 μM (black bar), U73343 10 μM (blue bar) and CPA 10 μM (orange bar not visible, marked with a red arrow). The numbers in the figure represent the number of cells studied. Statistical comparison between groups was performed using ANOVA test (* = p ≤ 0.05).
after histamine stimulation in WI-38 human lung fibroblasts is due to PLC activation and Ca²⁺ release from the ER.

**InsP₃R play an important role in histamine-evoked Ca²⁺ signaling in WI-38 human lung fibroblasts**

Having demonstrated that the ER Ca²⁺ stores contributes to histamine-evoked Ca²⁺ signals upon PLC activation, we evaluated the involvement of InsP₃R, which provides the main pathway for ER Ca²⁺ release in fibroblasts (Horie et al., 2014; Berra-Romani et al., 2020). The results obtained demonstrate that, after incubation for 20 min with 2-aminoethoxydiphenyl borate (2-APB, 50 μM), a drug widely used as InsP₃R inhibitor (Guzmán-Silva et al., 2015), the amplitude of histamine-evoked initial Ca²⁺ transient is significantly reduced compared to untreated cells, both in the presence (Figures 6A,C) and in the absence of extracellular Ca²⁺ (Figures 6B,C). In addition,
the number of oscillations was significantly decreased when fibroblasts were preincubated with 2-APB in normal Ca²⁺ (Figures 6A,D) and completely eliminated under 0Ca²⁺ conditions (Figures 6B,D). Even though 2-APB application during histamine-activated Ca²⁺ oscillations caused an immediate and transitory increase in [Ca²⁺]i, 2-APB subsequently erased the intracellular Ca²⁺ oscillations and plateau phase; this effect was reversible (Figure 6E). RyR could support InsP₃-induced intracellular Ca²⁺ oscillations through CICR, as reported in other cell types (Paltauf-Doburzynska et al., 2000). Nevertheless, the acute addition of caffeine (10 mM) mimicked the inhibitory effect of 2-APB by reversibly interrupting the oscillatory Ca²⁺ train (Supplementary Figure S1) in 92.3% of tested cells (60 out of 65 cells). This observation confirms that RyR, which are directly gated by caffeine (Pulina et al., 2010), do not contribute to histamine-induced intracellular Ca²⁺ waves in WI-38 fibroblasts and is consistent with the well-known phenomenon of InsP₃R inhibition by caffeine (Parker and Ivorra, 1991; Moccia et al., 2003). Taken together, these results suggest a strong involvement of InsP₃R in histamine-evoked Ca²⁺ transients in lung fibroblasts of the WI-38 cell line.

Extracellular Ca²⁺ influx contributes to histamine-induced intracellular Ca²⁺ signaling in WI-38 human lung fibroblasts

Next, we evaluated the contribution of extracellular Ca²⁺ to histamine-evoked Ca²⁺ signals by exposing the WI-38 fibroblasts to histamine in the absence of external Ca²⁺ to prevent Ca²⁺ entry across the plasma membrane (Berra-Romani et al., 2020). Histamine elicited an immediate increase in [Ca²⁺]i in the absence of extracellular Ca²⁺ in 66 of 69 cells (Figure 7A). Of note, exposure of WI-38 cells to 0Ca²⁺ conditions caused a significative reduction in basal [Ca²⁺]i (Figures 7A,B), which is consistent with the presence of a constitutive Ca²⁺ entry pathway (Zuccolo et al., 2018). When histamine (300 μM) was applied to fibroblasts under 0Ca²⁺ conditions, the peak and plateau amplitudes (Figure 7C), as well as the number of intracellular Ca²⁺
oscillations (Figure 7D), were significantly reduced compared to control conditions. In particular, Ca\(^{2+}\) oscillations rapidly run down in the absence of Ca\(^{2+}\) entry (Figure 7A). Furthermore, ongoing Ca\(^{2+}\) oscillations reversibly ceased upon removal of extracellular Ca\(^{2+}\) (Supplementary Figure S2) in 97.1% of tested cells (66 out of 68 cells). These results indicate that the peak and plateau amplitude as well as the number of Ca\(^{2+}\) oscillations evoked by histamine are due to both Ca\(^{2+}\) release from the ER and Ca\(^{2+}\) influx from the extracellular medium.

Blocking Ca\(^{2+}\) entry through VOCs does not affect histamine-evoked intracellular Ca\(^{2+}\) signals

There is evidence for a key role of L-type VOCs in TGF-β-induced Ca\(^{2+}\) signaling in human lung fibroblasts (Mukherjee et al., 2015). We, therefore, evaluated their involvement in histamine-induced intracellular Ca\(^{2+}\) signaling in WI-38 cells. We exploited a non-specific L-type VOC antagonist (nickel, 10 μM) (Figures 8A,C,E) and a specific VOC antagonist

![Image](https://via.placeholder.com/150)

**FIGURE 8** Blocking Ca\(^{2+}\) entry through VOCs does not affect histamine-evoked intracellular Ca\(^{2+}\) signals in WI-38 human lung fibroblasts. (A) Typical recording of histamine-evoked Ca\(^{2+}\) signals in the presence of nickel (10 μM). (B) Typical recording of histamine-evoked Ca\(^{2+}\) signals in the presence of nifedipine (10 μM). Comparison between groups was performed using the Mann-Whitney test (ns = not statistics differences between groups). (C) Mean ± SE of the peak and plateau amplitudes of the Ca\(^{2+}\) response to histamine (300 μM) in absence (dark green bar) or presence of the non-specific VOC inhibitor, nickel 10 μM (blue bar). (D) Mean ± SE of the peak and plateau amplitudes of the Ca\(^{2+}\) response to histamine (300 μM) in absence (dark green bar) or presence of the specific VOC inhibitor, nifedipine 10 μM (blue bar). (E) Mean ± SE of the number of oscillations recorded during the first 400 s after histamine application in presence (dark green bar) or presence of the unspecific VOC inhibitor, nickel 10 μM (blue bar). (F) Mean ± SE of the number of oscillations recorded during the first 400 s after histamine application in presence (dark green bar) or absence of the specific VOC inhibitor, nifedipine 10 μM (blue bar).
(nifedipine, 10 μM) (Figures 8B,D,F). The results show that there were no significant differences in the peak and plateau amplitudes, as well as in the number of oscillations, when Ca²⁺ entry through VOCs was inhibited with either nickel (Hobai et al., 2000) or nifedipine (Zhang et al., 2007), respectively.

Ca²⁺ influx through SOCs plays an important role in the plateau and oscillations of histamine-evoked Ca²⁺ transients in WI-38 lung fibroblasts

A recent investigation hinted at SOCE as the main Ca²⁺ entry pathway sustaining the Ca²⁺ response to chemical stimuli in human lung fibroblasts (Guzmán-Silva et al., 2015). Therefore, we evaluated the role of SOCs in the Ca²⁺ signal evoked by histamine in WI-38 fibroblasts. The pyrazole-derivative, BTP-2, which is widely employed to inhibit SOCE in non-excitable cells (Prakriya and Lewis, 2015; Moccia et al., 2016; Zhang et al., 2020), increases [Ca²⁺]i in human lung fibroblasts (Guzmán-Silva et al., 2015) and cannot be reliably used to assess SOCE involvement in histamine-evoked extracellular Ca²⁺ entry. In accord, Supplementary Figure S3 shows that BTP-2 (20 µM) induced an immediate elevation in [Ca²⁺]i upon application during the decay phase of the initial Ca²⁺ response to histamine. However, low micromolar doses of the trivalent cations, La³⁺ and Gd³⁺, can also selectively inhibit Orai channels, which provides the main pore-forming subunits of SOCs in both excitable and non-excitable cells (Prakriya and Lewis, 2015; Moccia et al., 2016; Zhang et al., 2020), including human lung fibroblasts (Guzmán-Silva et al., 2015; Vazquez-de-Lara et al., 2018). The results showed that the application of both La³⁺ (10 µM) and Gd³⁺ (10 µM) abrogated the plateau and Ca²⁺ oscillations in 79 and 40 cells exposed to La³⁺ and Gd³⁺, respectively (Figure 9A,B). This effect was reversible upon removal of La³⁺ or Gd³⁺. Intriguingly, the acute addition of La³⁺ and Gd³⁺ also caused a decrease in resting Fura-2 fluorescence ratio below the baseline, which suggests that Orai channels are involved in constitutive Ca²⁺ entry (see Figure 6B).

In summary, these results suggest a strong involvement of Ca²⁺ entry through SOCs in the histamine-evoked Ca²⁺ transient in lung fibroblasts of the WI-38 cell line.

Preliminary characterization of the Ca²⁺ handling machinery in in WI-38 fibroblast

No comprehensive information is available regarding the molecular composition of the Ca²⁺ handling toolkit in WI-38 fibroblasts. Therefore, we performed a preliminary qRT-PCR analysis of the main Ca²⁺-permeable channels that are known to shape the Ca²⁺ response to histamine in other cell types. We used the specific primers described in Table 1, while negative controls were carried out by excluding the reverse transcription reaction, as shown in (Negri et al., 2021). Figure 10 displays that WI-38 lung fibroblasts express the transcripts encoding for all the InsP₃R isoforms, i.e., InsP₃R1, InsP₃R2, and InsP₃R3, whereas, among the molecular players of the SOCE machinery, only STIM2 and Orai3 paralogues were present. In addition, the mRNAs encoding for RyR1 and most of the members of the Transient Receptor Potential Canonical (TRPC) subfamily, i.e., TRPC1, TRPC3, TRPC4, TRPC5, and TRPC6 (Negri et al., 2019), were also found (Figure 10). Therefore, these findings support the notion that the interaction between InsP₃R and SOC drive histamine-induced intracellular Ca²⁺ signals in WI-38 human adult lung fibroblasts.
Discussion

Pulmonary remodeling is the main long-term complication of asthma and occurs as a compensatory response to a persistent inflammatory state of the lower airway epithelium that leads to an irreversible restriction in the air flow, and is a result of a multi-step process known as “scarring.” Mast cells play a crucial pathogenic role in asthma by releasing the autacoid mediator histamine, which stimulates airway smooth muscle cell constriction and hyperplasia, and activates fibroblasts to acquire a contractile phenotype and support pulmonary remodelling. The molecular mechanisms whereby histamine promotes fibroblast proliferation and migration is still unclear, although preliminary evidence indicates that histamine-induced collagen gel contraction is mediated by an increase in \([\text{Ca}^{2+}]_i\) (Horie et al., 2014). Intriguingly, it has long been known that intracellular \([\text{Ca}^{2+}]_i\) signaling can adopt multiple patterns to orchestrate many of the cellular events that contribute to pulmonary remodeling, including fibroblast proliferation and transformation into myofibroblasts (Janssen et al., 2015). Herein, we provided the first clear-cut characterization of the signaling pathways that shape histamine-induced intracellular \([\text{Ca}^{2+}]_i\) signals in the WI-38 cell line, which is widely employed to study human lung fibroblasts. Our findings could pave the way towards an alternative strategy to target histamine signaling and thereby dampen airway remodeling in asthma.

Intracellular \([\text{Ca}^{2+}]_i\) signals regulate a broad spectrum of cellular processes by adopting diverse spatiotemporal dynamics, ranging from single \([\text{Ca}^{2+}]_i\) transient to repetitive \([\text{Ca}^{2+}]_i\) transients whose frequency depends on agonist strength (Smedler and Uhlén, 2014). The introduction of high-speed microfluorimetry and high-resolution image analysis techniques have allowed researchers to monitor changes in \([\text{Ca}^{2+}]_i\), in individual cells simultaneously. These techniques have revealed a surprising degree of heterogeneity in the \([\text{Ca}^{2+}]_i\) responses to the same agonist generated even by cells in the same field of view. Herein, we report that histamine evoked complex \([\text{Ca}^{2+}]_i\) waveforms in WI-38 fibroblasts that could be classified according to the following patterns: 1) peak, 2) peak and oscillations, 3) peak plateau and oscillations, and 4) peak and plateau. Spatial and temporal heterogeneity of the \([\text{Ca}^{2+}]_i\) signature can be a hallmark of the \([\text{Ca}^{2+}]_i\) response evoked by both mechanical (Berra-Romani et al., 2008; Jing et al., 2013) and chemical cues (Dupont and Combettes, 2016; Wacquier et al., 2019). A recent investigation showed that, in normal human lung fibroblasts (NHLF), beractant (a natural surfactant) induced distinct patterns of intracellular \([\text{Ca}^{2+}]_i\) signals, each comprising an initial \([\text{Ca}^{2+}]_i\) spike that could be followed either by a) transient, by repetitive \([\text{Ca}^{2+}]_i\) oscillations, c) a sustained \([\text{Ca}^{2+}]_i\) plateau or d) a sustained plateau overlapped by repetitive \([\text{Ca}^{2+}]_i\) oscillations (Guzmán-Silva et al., 2015). Similarly, ATP could elicit either a single \([\text{Ca}^{2+}]_i\) transient or recurring \([\text{Ca}^{2+}]_i\) oscillations in NHLF (Janssen et al., 2015), whereas an heterogenous array of agonist-induced intracellular \([\text{Ca}^{2+}]_i\) signals has been described in fibroblasts deriving from other tissues (Chen et al., 2010; Kemény et al., 2013; Lembong et al., 2015). As expected (Ong et al., 2019), the diverse \([\text{Ca}^{2+}]_i\) dynamics and the number of oscillations depend on histamine concentration, with the lower concentration being dominated by the peak pattern and the higher concentration being dominated by the peak, plateau
and oscillations pattern. In accord, low doses of histamine are predicted to elicit only intracellular Ca²⁺ release, while the Ca²⁺ response to higher doses can also involve extracellular Ca²⁺ entry (Ong et al., 2019). Likewise, a recent investigation showed that histamine was more eager to induce 1 or 2 Ca²⁺ spikes at low picomolar doses, whereas the number and frequency of repetitive Ca²⁺ transients progressively increased with histamine concentration (up to 300 µM) in human microvascular endothelial cells (Berra-Romani et al., 2020). The non-cumulative concentration-response relationship showed that the Ca²⁺ signal evoked by histamine in WI-38 human lung fibroblasts present an EC₅₀ value of 4.96 µM, a maximum concentration of 300 µM, and a threshold concentration of 100 nM. Similarly, histamine induced collagen gel contraction and proliferation in, respectively, primary cultured human lung fibroblasts (Horie et al., 2014) and IMR-90 adult lung fibroblasts (Kunzmann et al., 2007) within a concentration range spanning from 100 nM up to 100 µM. Moreover, the pro-migratory effect of histamine on human lung fetal fibroblasts appeared at a threshold dose of 100 nM (Kohyama et al., 2010). These studies concur with the evidence that H1R mediates histamine-induced proliferation, migration, and collagen gel contraction in human lung fibroblasts. In agreement with these observations, pharmacological manipulation showed that histamine generates a complex increase in [Ca²⁺], in WI-38 fibroblasts mainly through H1R and, to a lesser extent, through H2R. Furthermore, short-term exposure of the cells to histamine reduced the responsiveness to subsequent applications of the agonist, which is a hallmark of H1R signalling (Smit et al., 1992). Homologous desensitization of H1R has also been reported in smooth muscle preparations (Leurs et al., 1990; Leurs et al., 1991), HeLa cells (Smit et al., 1992), and human gingival fibroblasts (Gutiérrez-Venegas and Rodríguez-Pérez, 2012). Conversely, H3R and H4R do not seem to play a crucial role in the onset of the Ca²⁺ signal. These results were supported by the evidence that histamine-evoked intracellular Ca²⁺ waves were not altered by the PT-dependent ribosylation of the Goαi subunit, which triggers the signalling cascades activated downstream of both H3R and H4R (Seifert et al., 2013). Consistent with our observations, Horie et al. (2014) previously reported the involvement of H1R in histamine-induced collagen gel contraction in primary cultured lung fibroblasts, whereas H2R could be responsible for a small Ca²⁺ response occurring in the presence of diphenhydramine, a specific H1R-antihistamine.

The mechanisms that control the mobilization of cytosolic Ca²⁺ are key to the regulation of numerous eukaryotic cell functions (Clapham, 2007). Therefore, after identifying the HR subtype responsible for histamine-evoked Ca²⁺ signaling, we set out to dissect the molecular underpinnings of the Ca²⁺ transient. H1R is a GqPCR that can signal an increase in [Ca²⁺], by stimulating PLCβ to synthesize InsP₃ and trigger ER Ca²⁺ release through InsP₃R (Berra-Romani et al., 2012; Seifert et al., 2013; Berra-Romani et al., 2020). In accord, the Ca²⁺ response to histamine still occurred in the absence of extracellular Ca²⁺, although it rapidly run down after 1-3 Ca²⁺ spikes. Furthermore, histamine-evoked intracellular Ca²⁺ signals were strongly reduced by blocking PLCβ activity with U73122, but not its inactive analog, U73343. Furthermore, the initial Ca²⁺ peak was significantly reduced as compared to control, i.e., untreated, cells upon inhibition of InsP₃R with 2-APB. This inhibitory effect was observed both in the presence and in the absence of extracellular Ca²⁺. 2-APB significantly decreased the number of oscillations under normal Ca²⁺ conditions, while it completely erased the spiking response under 0Ca²⁺ conditions. Of note, inhibition of InsP₃Rs with 2-APB, despite decreasing the amplitude of the Ca²⁺ transients, did not completely eliminate histamine-induced Ca²⁺ signals, as previously reported both in fibroblasts (Horie et al., 2014) and in other cell types (Berra-Romani et al., 2020). Unlike histamine, the Ca²⁺ response to beractant in WI-38 fibroblasts was fully abrogated by 2-APB (Guzmán-Silva et al., 2015). This discrepancy can be explained by invoking several hypotheses. First, the ability of 2-APB to penetrate the cell membrane may significantly vary among different cell types: this feature could explain the high sensitivity to 2-APB observed in some cells, but not in others (Souslbs and Wojcikiewicz, 2002). Second, the degree of 2-APB-dependent inhibition could depend on the histamine concentration employed to characterize the Ca²⁺ response in WI-38 lung fibroblasts. For instance, early work carried out in HeLa cells showed that 100 µM 2-APB was able to completely inhibit the ATP-evoked Ca²⁺ response at all the tested concentrations, while histamine-evoked Ca²⁺ signals were only slightly reduced at high agonist doses (i.e., 100 µM) (Peppiatt et al., 2003). It is likely that a higher concentration of 2-APB is required to block InsP₃R recruited by histamine in WI-38 lung fibroblasts. However, we did not increase 2-APB concentration to avoid the concentration-dependent side-effects that have been associated to this powerful InsP₃R inhibitor, such as SOCE inhibition and SERCA modulation (Gambardella et al., 2021). The primary role of InsP₃R in the Ca²⁺ response to histamine was further corroborated by caffeine, which reversibly inhibited, rather than enhancing, histamine-induced intracellular Ca²⁺ oscillations. At the concentration employed in the present investigation, caffeine can either stimulate RyR (Pullina et al., 2010) or inhibit InsP₃R (Parker and Ivorra, 1991; Moccia et al., 2003). Therefore, the blocking effect of caffeine further confirms that InsP₃R are the main responsible for the rhythmic ER Ca²⁺ release induced by caffeine in WI-38 fibroblasts.

While the complex increase in [Ca²⁺], is triggered by ER Ca²⁺ mobilization through InsP₃R, the Ca²⁺ response is maintained over time by extracellular Ca²⁺ entry. In accord, removal of external Ca²⁺ resulted in the decrease of the Ca²⁺ peak and Ca²⁺ plateau amplitudes, and in the number of Ca²⁺ oscillations evoked by histamine. These findings concur with previous studies showing that Ca²⁺ influx through the plasma membrane sustains intracellular Ca²⁺
oscillations induced by H1R stimulation in several cell types, including cerebrovascular endothelial cells (Berra-Romani et al., 2020), HeLa cells (Sauvé et al., 1991), and vascular smooth muscle cells (Espinosa-Tanguma et al., 2011). SOCE is activated upon depletion of the ER Ca\(^{2+}\) pool and represents the Ca\(^{2+}\) entry pathway that sustains the Ca\(^{2+}\) signal induced by agonists stimulation in fibroblasts from different tissues, including human mammary gland (Sadaras et al., 2021b), human heart (Chung et al., 2021), human skin (Wu et al., 2019), and human lungs (Guzmán-Silva et al., 2015). It has been nicely documented that low (1–10) micromolar doses of the trivalent cations, La\(^{3+}\) and Gd\(^{3+}\), plug the access to the Orai channel inner pore, thereby specifically inhibiting SOCE (Prakriya and Lewis, 2015; Mocia et al., 2016; Zhang et al., 2020). In accord, the application of either SOC blocker at 400 s after histamine application completely abolished the oscillations and suppressed the Ca\(^{2+}\) plateau in WI-38 lung fibroblasts. Similar results were achieved by the acute addition of 10 µM La\(^{3+}\) and 10 µM Gd\(^{3+}\) on the long-lasting elevation in [Ca\(^{2+}\)], evoked in the same cells by beractant (Guzmán-Silva et al., 2015). VOC represent an alternative pathway for extracellular Ca\(^{2+}\) entry in NHLF, as shown for the intracellular Ca\(^{2+}\) oscillations induced by transforming growth factor β (Mukherjee et al., 2015). However, two structurally unrelated VOC inhibitors, i.e., nickel and nifedipine, did not affect histamine-evoked intracellular Ca\(^{2+}\) signals in WI-38 cells. We hypothesize that histamine-dependent SOC activation does not depolarize the membrane potential to such an extent to induce VOC activation.

A preliminary qRT-PCR analysis of the Ca\(^{2+}\) handling machinery confirmed that WI-38 fibroblasts express all the three known InsP\(_3\)R isoform and two of the molecular components required to activate SOCE, i.e., STIM2 and Orai3. These data are therefore consistent with the results provided by the pharmacological manipulation of the Ca\(^{2+}\) response. Conversely, all Orai and STIM isoforms were detected in human cardiac fibroblasts (Cendula et al., 2021), in which they support spontaneous Ca\(^{2+}\) oscillations (Chen et al., 2010). Intriguingly, Orai3 can be directly activated by 50 µM 2-APB independent from ER Ca\(^{2+}\) store depletion (Zhang et al., 2020), which might explain the immediate rise in [Ca\(^{2+}\)], that occurs upon 2-APB application in the presence (see Figure 6F) but not in the absence (not shown) of extracellular Ca\(^{2+}\). RyR1 transcript was also found, but it is unlikely to contribute to histamine-evoked intracellular Ca\(^{2+}\) signals, as shown by the inhibitory effect of caffeine. WI-38 fibroblasts also express the transcripts encoding for TRPC1-TRPC6, but the pharmacological sensitivity of histamine-evoked Ca\(^{2+}\) entry to 10 µM La\(^{3+}\) and Gd\(^{3+}\) argues against the involvement of TRPC isoforms. Moreover, a recent investigation demonstrated that TRPC channels do not support SOCE in primary murine lung fibroblasts (Bendiks et al., 2020). Conversely, TRPC channels, which present a single-channel conductance that is 1000-fold larger than Orai3, could be activated by transforming growth factor β and thereby lead to VOC activation via strong membrane depolarization (Mukherjee et al., 2015). Quite surprisingly, this was the first molecular characterization of the Ca\(^{2+}\) toolkit in human pulmonary fibroblasts. The selective expression of STIM2 as ER Ca\(^{2+}\) sensor might explain the rapid fall in resting [Ca\(^{2+}\)], observed upon removal of external Ca\(^{2+}\). In accord, STIM2 is activated only by a mild depletion of the ER Ca\(^{2+}\) store and can drive the activation of a constitutive Ca\(^{2+}\) influx (Brandman et al., 2007). In agreement with this hypothesis, preliminary evidence showed that µM La\(^{3+}\) and Gd\(^{3+}\) reduced the basal Ca\(^{2+}\) entry, thereby suggesting that SOC also support the resting Ca\(^{2+}\) permeability of WI-38 fibroblasts (Sanchez-Collado et al., 2020). It is worth of pointing out that the most frequent Ca\(^{2+}\) patterns evoked by high doses of histamine, i.e., mode 2) peak-oscillations, 64.7%, and mode 3) peak-plateau-oscillations, 13.39%, entail the occurrence of intracellular Ca\(^{2+}\) oscillations. In agreement with this observation, STIM2 and Orai3 can enhance the percentage of cells showing intracellular Ca\(^{2+}\) oscillations upon GqPCR stimulation (Yoast et al., 2020; Emrich et al., 2021). Furthermore, mathematical modelling has shown that, because of their distinct sensitivity to cytosolic Ca\(^{2+}\), InsP\(_3\)R3 may provide a constant release of Ca\(^{2+}\) that stimulates InsP\(_3\)R1 and InsP\(_3\)R2 to rhythmically release ER stored Ca\(^{2+}\), whereas SOCE maintains the Ca\(^{2+}\) response by ensuring ER Ca\(^{2+}\) refilling (Dupont and Croisier, 2010; Dupont, 2014). However, the periodic Ca\(^{2+}\) transients transition into a sustained plateau either when InsP\(_3\)R3 expression increases (Okumura et al., 2022) or when ER Ca\(^{2+}\) release through InsP\(_3\)R3 is enhanced by the tight coupling with the ER-embedded protein, Jaw1 (Okumura et al., 2022). Therefore, the molecular assortment of the distinct STIM/Orai and InsP\(_3\)R isoform, as well as cell-to-cell variability in their expression, subcellular distribution, or posttranslational regulation, could contribute to pattern a heterogeneous array of Ca\(^{2+}\) signatures in WI-38 adult lung fibroblast (Ishida et al., 2014; Guzmán-Silva et al., 2015; Bartok et al., 2019; Wilson et al., 2020). Conversely, cell cycle asynchrony is an unlikely explanation of the cell-to-cell heterogeneity of histamine-evoked Ca\(^{2+}\) waves because our experiments were performed in fibroblasts devoid of serum for 48 h, which causes cell cycle arrest in G0 phase (Santella, 1998). Similarly, previous studies in fibroblasts and other cell types have reported that this variability in the intracellular Ca\(^{2+}\) dynamics is not due to cell cycle asynchrony (Ambler et al., 1988; Byron and Villereal, 1989; Dragoni et al., 2011; Guzmán-Silva et al., 2015; Okumura et al., 2022).

In conclusion, the present investigation showed that histamine induces a dose-dependent increase in [Ca\(^{2+}\)], in the widely employed human pulmonary fibroblast cell line, WI-38. The Ca\(^{2+}\) signal is mainly triggered by H1R and can adopt multiple signatures, the most common of which encompasses intracellular Ca\(^{2+}\) oscillations, which have long been known to stimulate gene expression, proliferation, contraction and migration in human pulmonary fibroblasts (Janssen et al., 2015). The Ca\(^{2+}\) response to histamine is triggered by ER Ca\(^{2+}\) release through InsP\(_3\)R and maintained over time by SOCE activation. These data suggest that the Ca\(^{2+}\) handling machinery could provide an alternative molecular target to prevent the pernicious effects of histamine on lung fibroblasts in asthmatic patients, as recently suggested also
for pulmonary hypertension (Bikou et al., 2022), Streptococcus pneumoniae-induced lung injury (Ali et al., 2022), and asthma itself (Johnson et al., 2022). Much research remains therefore to be done to assess this issue, although the work presented here provides valuable information for understanding the mechanisms that regulate histamine-evoked Ca\(^{2+}\) signaling in lung fibroblasts. A limitation of the present study is that we did not use lung fibroblasts from an asthmatic model or from patients with asthma. Future work will have to compare the effect generated by histamine in lung fibroblasts from normal airways and in lung fibroblasts from asthmatic airways.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

RB-R and FM conceived and directed the project in collaboration with MG-C, AI-M, UL, and JA-C. AV-G, JS-G, NC-S, MS, GP, and EH-A performed the experiments and analyzed the data. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2022.991659/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Effect of caffeine on histamine-evoked Ca\(^{2+}\) signals in WI-38 human lung fibroblasts. Typical recording of histamine-evoked Ca\(^{2+}\) signals and effect of the application of 10 mM caffeine.

SUPPLEMENTARY FIGURE S2
Effect of extracellular Ca\(^{2+}\) removal on histamine-evoked Ca\(^{2+}\) oscillations in WI-38 human lung fibroblasts. Typical recording of histamine-evoked Ca\(^{2+}\) oscillations and effect of extracellular Ca\(^{2+}\) removal.

SUPPLEMENTARY FIGURE S3
Effect of BTP-2 on histamine-evoked Ca\(^{2+}\) signals in WI-38 human lung fibroblasts. Typical recording of histamine-evoked Ca\(^{2+}\) signals and effect of BTP-2 (n = 76).
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