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Treatment of mast cells with carbon dioxide suppresses degranulation via a novel mechanism involving repression of increased intracellular calcium levels

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Abstract

Background: Intranasal noninhaled delivery of carbon dioxide (CO_2) is efficacious in the symptomatic treatment of seasonal allergic rhinitis. The goal of this study was to determine whether and how 100% CO_2 inhibits mast cell degranulation, thereby possibly contributing to the reduction of symptoms in seasonal allergic rhinitis.
Methods: Peritoneal mast cells isolated from rats and labelled with sulforhodamine-B (SFRM-B) were used to determine whether CO_2 treatment could block mast cell degranulation and histamine release in response to 48/80. In addition, the effect of CO_2 on intracellular calcium levels in unstimulated and stimulated mast cells was determined by fluorescent microscopy.
Results: Treatment with 48/80 caused >90% of mast cells containing SFRM-B to degranulate, resulting in a marked decrease in the fluorescent intensity within the mast cells, and simultaneously causing a significant increase in histamine release. Significantly, the stimulatory effect of 48/80 on fluorescent intensity and histamine levels was greatly inhibited (>95%) to near control levels by pretreatment with 100% CO_2. Treatment with 48/80 also caused a robust transient increase in intracellular calcium, whereas pretreatment with CO_2 repressed the increase in calcium (>70%) in response to 48/80.
Conclusions: Results from this study provide the first evidence of a unique regulatory mechanism by which CO_2 inhibits mast cell degranulation and histamine release by repressing stimulated increases in intracellular calcium. Thus, our data provide a plausible explanation for the reported therapeutic benefit of noninhaled intranasal delivery of 100% CO_2 to treat allergic rhinitis.

Mast cells play a pivotal role in initiating and maintaining a wide range of allergic and nonallergic inflammatory diseases including allergic rhinitis, rhinosinusitis, asthma, and possibly migraine (1–3). Allergic rhinitis and rhinosinusitis were prevalent in 10–30% of adults and as many as 40% of children (4). It is estimated that the prevalence of allergic rhinitis in the United States is >60 million people and is reported to be increasing (5). Most of the current therapies for treating rhinitis target the downstream effects of mast cell degranulation and inflammation in general (6). New therapies that prevent mast cell degranulation and thus release of inflammatory mediators that can cause activation of trigeminal nerves would be beneficial for managing allergic rhinitis.

Towards this end, noninhaled intranasal delivery of 100% CO_2 effectively relieves the primary symptoms of seasonal allergic rhinitis (7). Results from phase II clinical trials provided evidence that noninhaled intranasal delivery of 100% CO_2 is an effective and safe abortive treatment for seasonal allergic rhinitis. Results from a single-centre, randomized, double-blind, placebo-controlled, parallel group study showed two 60- s intranasal CO_2 treatments resulted in rapid (10 min) and sustained (24 h) relief of seasonal allergic rhinitis symptoms. Although the use of CO_2 is therapeutically beneficial, the cellular mechanism by which CO_2 treatment relieves clinical symptoms of allergic rhinitis are not well understood but could possibly involve inhibition of mast cell degranulation. Mast cell degranulation and subsequent release of inflammatory molecules are implicated in initiating and maintaining allergic and nonallergic rhinitis and rhinosinusitis (8–10). Typically, *in vivo*, there are two pathways in which

mast cell activation occurs. FcεRI-mediated mast cell activation occurs when antigen-bound IgE immunoglobulins bind to IgE receptors on the surface of mast cells. This event initiates a signalling cascade leading to degranulation and release of inflammatory molecules, including cytokines, TNF-α, chemokines and histamine (11–16). Mast cell activation can also be mediated by other inflammatory molecules such as neuropeptides including substance P, neurokinin A, bradykinin and VIP (17–20). Neuropeptide receptor-mediated mast cell activation occurs when neuropeptides bind G-protein coupled receptors, which initiates a signalling cascade leading to degranulation and release of the same inflammatory molecules released in response to IgE receptor activation. Receptor-mediated mast cell activation and degranulation are preceded by a spike in intracellular calcium, followed by activation of store-operated calcium channels (21–25). The more prolonged rise in intracellular calcium in response to the opening of store-operated calcium channels is required for vesicle docking and exocytosis of mast cell granules (25–27).

In a prior study, we provided evidence that treatment of trigeminal ganglion neurons, which provide sensory innervation of the nasal mucosa, with 100% CO_2 repressed stimulated neuropeptide secretion by inhibiting the increases in intracellular calcium (28). In this study, the mechanism by which 100% CO_2 inhibits mast cell degranulation was investigated using isolated rat peritoneal mast cells. We found that treatment with 100% CO_2 greatly repressed mast cell degranulation and histamine release in response to the secretagogue 48/80 via a mechanism that prevented the normal rise in intracellular calcium observed in response to 48/80. Based on our findings, we propose that a primary therapeutic benefit of noninhaled intranasal delivery of 100% CO_2 to alleviate symptoms of allergic rhinitis probably involves blocking mast cell degranulation.

Materials and methods

Animals

Young male Sprague–Dawley rats (200–250 g; Charles River Laboratories, Wilmington, MA, USA) were housed in plastic cages on a 12-h light/dark cycle with unrestricted access to food and water. Experiments were approved by the Institutional Animal Care and Use Committee at Missouri State University. A concerted effort was made to reduce the suffering and number of animals used in this study.

Mast cell preparation

Rats were killed by CO_2 asphyxiation and decapitated. HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)-buffered saline (5 ml; HBS; 22.5 mM HEPES,135 mM NaCl, 3.5 mM KCl, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, 3.3 mM glucose, and 0.1% BSA, pH 7.4) was injected in the peritoneal cavity and the abdomen was massaged for 1 min. Approximately 3 ml of peritoneal eluate cells were harvested, spun at 100 ***g*** for 3 min and resuspended in 3 ml of fresh HBS. Cells (200 μl) were plated on 13 mm plastic coverslips coated with poly-D-lysine in a 24-well tissue culture plate. Cells were incubated at 37°C and ambient CO_2 levels for 10 min to facilitate attachment of cells to the coverslips. Once the cells had attached, an additional 300 μl of HBS was added to each well and cells returned to the incubator.

Mast cell identification

Following a 15 min incubation, rat peritoneal cells were stained with toluidine blue (0.1% in phosphate-buffered saline; Sigma-Aldrich, St. Louis, MO, USA) for 5 min at 37°C. Mast cells were identified using phase and light microscopy at 400× magnification. For the degranulation studies, cells were incubated in HBS (control) or HBS containing the secretagogue compound 48/80 (methoxyphenylethyl-methyl-amine and formaldehyde; 1 μg/ml in HBS; BIOMOL, Plymouth Meeting, PA, USA) for 15 min at 37°C. Degranulation of mast cells was recorded in real time using phase microscopy with a Hamamatsu C4742-95 camera mounted on a Leica DMI 6000 B microscope equipped with IP LAB software, version 4.0 (North Central Instruments, St Louis, MO, USA).

Degranulation of fluorescently labelled mast cells with 48/80

Fluorescent dye experiments were based on a previously published method for labelling mast cells (29). Briefly, to induce mast cells to take up the fluorescent dye sulforhodamine-B (SFRM-B; Invitrogen, Carlsbad, CA, USA), cells were incubated in 500 μl of HBS containing 200 μg/ml SFRM-B and 0.25 μg/ml of 48/80 for 15 min at 37°C. Cells were then washed with HBS to remove any residual dye or 48/80 present in the extracellular medium. To induce the degranulation, SFRM-B-loaded cells were treated for 15 min at 37°C with a higher concentration of 48/80 (1 μg/ml). For the time course study, sequential pictures of the same field of mast cells were taken continuously every 5 s for 15 min after exposure to 1 μg/ml 48/80. Fluorescent and phase pictures were captured as described earlier. Each condition was repeated in a minimum of six independent experiments.

Effects of CO_2 on mast cell degranulation

Initially, mast cells labelled with SFRM-B were incubated in 500 μl of isohydric HEPES-buffered saline (IH HBS; 22.5 mM HEPES, 135 mM NaCl, 3.5 mM KCl, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, 3.3 mM glucose, 100 mM NaH_2PO_4, 100 mM $NaHCO_3$, and 0.1% BSA, pH 7.4). As described in a previous study (28), an isohydric buffer was used to prevent a decrease in external pH in the media in response to CO_2. Cells were incubated in IH HBS alone (control) or IH HBS containing a degranulating concentration of 48/80 (1 μg/ml) for 15 min at 37°C, and the change in dye intensity measured. To determine the effect of 100% CO_2 exposure on mast cells, labelled mast cells attached to 13 mm coverslips were placed in a saturated chamber, and 80 μl of IH HBS was placed on the coverslip. Mast cells were exposed to 100% CO_2 for 5 min then placed in IH HBS for 15 min at

37°C (20 min total time) or exposed to 100% CO_2 for 5 min prior to the addition of IH HBS containing 1 μg/ml 48/80 for 15 min at 37°C (20 min total time). The time of CO_2 exposure was based on data from our previous study utilizing a nonisohydric media with a pH-sensitive dye, which provided evidence that exposure to 100% CO_2 for 5 min was sufficient to permeate and lower the pH of the media (80 μl) (28). Images were collected from a minimum of six separate slides from each condition. Both fluorescent and phase images of mast cells were captured using an Olympus DP70 camera mounted on an Olympus BX41 fluorescent microscope, and image analysis was performed using Olympus MicroSuite Five image processing software version 2.2 (Center Valley, PA, USA).

Intensity measurements of mast cell fluorescence

Fluorescent images from the CO_2 studies were converted to greyscale prior to analysis. Average grey value was measured in a fixed circular region of interest positioned directly over each individual mast cell identified in a corresponding phase image of the same field of cells. Data are reported as an average ± SEM greyscale intensity of mast cells for each condition (CON, 48/80, CO_2, and CO_2 + 48/80). Average grey values were measured in mast cells from five fields of view per slide, and a minimum of six separate slides from each condition. Reported numbers are the average of counts obtained by two laboratory staff blinded to the experimental design. Each experimental condition was repeated in at least three independent experiments.

Effects of CO_2 on histamine release

Initially, mast cells were incubated in 500 μl of IH HBS alone (control) or IH HBS containing 48/80 (1 μg/ml) for 15 min at 37°C. To determine the effect of 100% CO_2 exposure, mast cells in IH HBS were exposed to 100% CO_2 for 5 min then placed in IH HBS for 15 min at 37°C (20 min total time) or exposed to 100% CO_2 for 5 min prior to the addition of IH HBS containing 1 μg/ml 48/80 for 15 min at 37°C (20 min total time). Media from each of the samples were collected and assayed for histamine using a commercially available histamine enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI, USA).

Effects of CO_2 on 48/80-induced elevation of intracellular calcium levels

Intracellular calcium levels in cultured rat peritoneal mast cells were measured essentially as described in other studies (30, 31). Cells were incubated in HBS and 5 μM fura-2 AM (Invitrogen) for 45 min at 25°C and then incubated in IH HBS. Absorbance in mast cells was determined at 340 and 380 nm every 2–8 s for a minimum of 10 min at 37°C. In all the studies, basal intracellular calcium levels were obtained for a minimum of 2 min before the treatment and for as long as 20 min following the final treatment. Initially, 1 μg/ml of 48/80 was added to cultures to measure peak calcium response to stimulus. In experiments with CO_2, cultures were initially treated with 100% CO_2 for 5 min in IH HBS prior to the addition of 48/80. Data are reported as the ratio of 340/380 nm wavelength values corresponding to bound versus unbound intracellular calcium. Calcium measurements were performed using a Hamamatsu camera mounted on a Leica DMI 6000 B fluorescent microscope with IP LAB software, version 4.0. Results are presented as the mean ± SEM peak amplitude for a minimum of 6–9 cells being analysed for each experimental condition, which was repeated in a minimum of three independent experiments performed in duplicate.

Statistical analysis

Statistical analyses for both mast cell fluorescence intensities and calcium levels were performed using one-way ANOVA Tukey and Schefe analysis. Differences were considered statistically significant at $P < 0.05$. Statistical analysis for histamine levels were performed using the nonparametric Mann–Whitney *U*-test. Differences were considered statistically significant at $P < 0.05$. All the statistical tests were performed using SPSS Statistical Software, Release 16 (Chicago, IL, USA).

Results

Mast cell identification and stimulation with 48/80

To confirm the presence of mast cells within peritoneal eluates, cultures were stained with toluidine blue. When stained with toluidine blue, mast cell granules undergo a metachromatic shift in colour from blue to a deep red/purple. As seen in Fig. 1A, many mast cells were identifiable in the peritoneal eluate. On the basis of our cell counts, the mast cell population comprises roughly 10–15% of the total peritoneal eluate. Mast cells identified using phase microscopy have a reflective halo with dense vesicles visible inside the cell (Fig. 1B). To further identify and confirm the normal function of the isolated mast cells, compound 48/80 at 1 μg/ml was added to the same field of mast cells as seen in Fig. 1B for 15 min. Exposure to 48/80 for 15 min caused labelled mast cells to degranulate (Fig. 1B').

Time-lapse degranulation of mast cells with compound 48/80

The fluorescent mast cell experiments are based on the premise that when treated with a lower concentration of 48/80 (0.25 μg/ml), mast cells are stimulated sufficiently to take up compounds in the extracellular medium, such as the fluorescent dye SFRM-B, by the process of endocytosis. Upon exposure to a higher dose of 48/80 (1 μg/ml), the mast cells degranulate and release SFRM-B. To demonstrate that mast cells temporally degranulate and release SFRM-B when treated with 1 μg/ml 48/80, a real-time, time-lapse degranulation experiment was performed using mast cells containing SFRM-B. Upon exposure to 1 μg/ml 48/80, degranulation, as seen in the phase images, was observed in several mast cells as quickly as 10 s after the addition of 48/80 but only a small

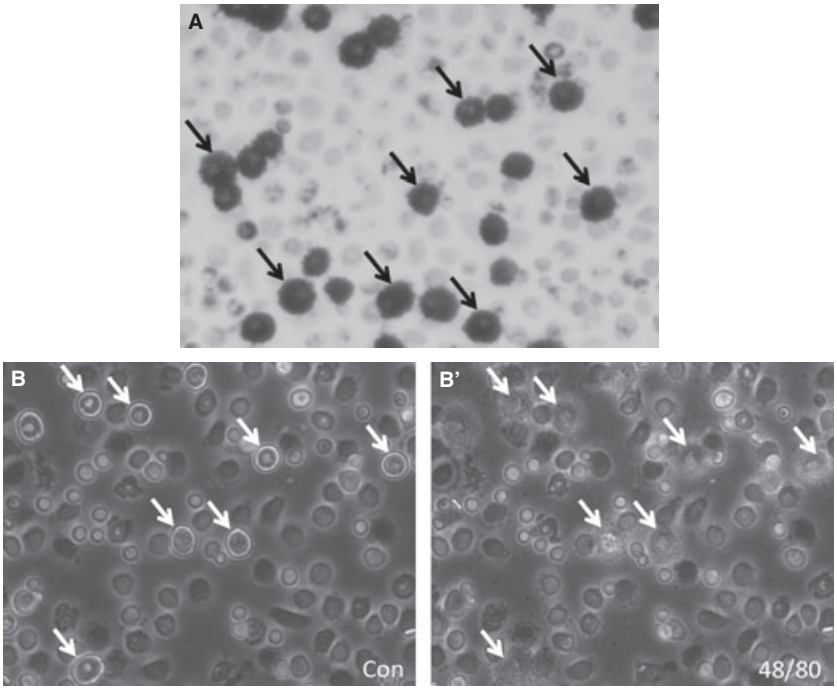

Figure 1 The secretagogue 48/80 causes degranulation of peritoneal mast cells. Mast cells (black arrows) from peritoneal eluate were stained with toluidine blue and visualized under light microscopy at 400× magnification (A). Numerous mast cells are readily identified in a field of peritoneal cells. Similarly, numerous mast cells (white arrows) are identifiable by a reflective halo with dense vesicles inside the cell using phase microscopy at 400× magnification (B). The same field of mast cells was exposed to 1 μg/ml 48/80 for 15 min to promote mast cell degranulation (B').

amount of the internalized SFRM-B was released even over a period of 1 min (Fig. 2). However, by 15 min, the majority of the internalized dye had been released and any remaining dye was barely detectable in the mast cells. These changes are more readily observed in a single representative mast cell from the time-lapse degranulation experiment (Fig 2B). In that cell, a decrease in fluorescent intensity was seen as early as 10 s after the addition of 48/80 when compared to control levels prior to the addition of the secretagogue. Almost all the dye had been released 15 min after treatment with 48/80. As a control, it was found that SFRM-B by itself did not cause mast cell degranulation as determined using phase or fluorescent microscopy (data not shown). In addition, the decrease in dye intensity in the mast cells was not attributed to photobleaching because no change in fluorescent intensity was observed in unstimulated labelled mast cells (data not shown). Thus, while degranulation is observed within seconds after the addition of 48/80, 15 min was chosen as the endpoint in subsequent studies because most of the dye was released from the cell at that time point.

Effect of CO_2 on 48/80-induced mast cell degranulation

To determine whether CO_2 treatment could inhibit mast cell degranulation, mast cells labelled with SFRM-B were left untreated in IH HBS for 15 min, incubated IH HBS supplemented with 48/80 (1 μg/ml) for 15 min, pretreated with 100% CO_2 for 5 min then placed in IH HBS for 15 min (20 min total), or pretreated with 100% CO_2 for 5 min and then incubated with 1 μg/ml 48/80 for 15 min (20 min total). For each condition, cells were initially visualized under phase microscopy to confirm mast cell morphology (Fig. 3). The same field of mast cells was then observed using fluorescent microscopy to determine the effect of compound 48/80 and treatment with 100% CO_2 on mast cell degranulation. In the control condition, many of the mast cells identified in the phase picture also contain SFRM-B. When labelled mast cells were exposed to 1 μg/ml 48/80, stimulated cells released internalized SFRM-B resulting in a lower level dye intensity in the cells and increased dye in the medium (Fig. 3). As a control, mast cells pretreated with 100% CO_2 for 5 min appeared similar to the control condition in which most of the SFRM-B-loaded mast cells still contained the dye. Thus, CO_2 treatment did not cause observable degranulation of labelled mast cells. In addition, a trypan blue exclusion assay was performed to test cell viability when mast cells were exposed to 100% CO_2 for 5 min. CO_2 treatment did not cause a decrease in mast cell viability when compared to untreated control (viability >97% for both conditions; data not shown). Importantly, pretreatment with CO_2 prior to the addition of 48/80 prevented mast cell degranulation and increased levels of the dye in the medium. This finding provides evidence that CO_2 is inhibiting 48/80-induced degranulation of SFRM-B-loaded mast cells.

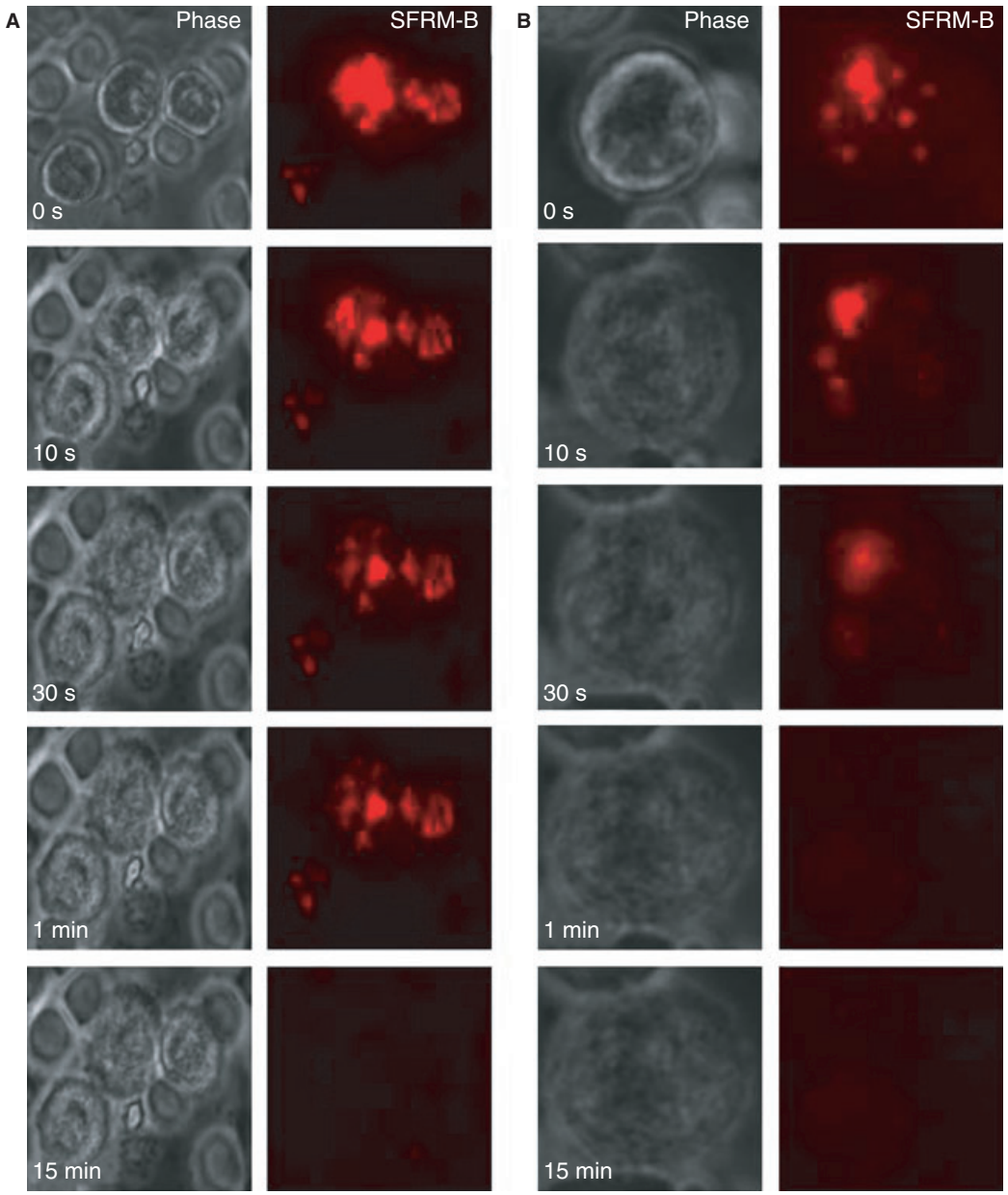

Figure 2 Time-lapse degranulation of mast cells in response to compound 48/80. An image (650×) of a group of mast cells (Fig. 2A) and an enlarged image of a single mast cell (Fig. 2B) loaded with the fluorescent dye SFRM-B and then treated with 48/80 are shown. Both phase and fluorescent microscopy images are shown at several time points after the addition of 1 μg/ml 48/80.

Intensity measurements of mast cell fluorescence

For each condition, mast cells were visualized under fluorescent and phase microscopy to observe effects of CO_2 on 48/80-induced mast cell degranulation. Multiple images of each condition obtained from several independent experiments were collected, and fluorescent intensities of each image were converted to greyscale. The average relative fluorescent intensity value for labelled mast cells from the control condition was 125 (set as 100%; Fig. 4). The average relative fluorescent intensity in mast cells exposed to 1 μg/ml of 48/80 for 15 min was greatly reduced to ~19% of control levels. In contrast, when mast cells were exposed to 100% CO_2 alone for 5 min, the average relative intensity (~102%) was close to that of control levels. Similarly, when mast cells were pretreated with 100% CO_2 for 5 min prior to exposure to 1 μg/ml 48/80, the relative intensity in the mast cells (~101%) remained near control levels. Thus, our results from the fluorescent dye experiments demonstrate that treatment of mast cells to 100% CO_2 suppresses 48/80-induced mast cell degranulation.

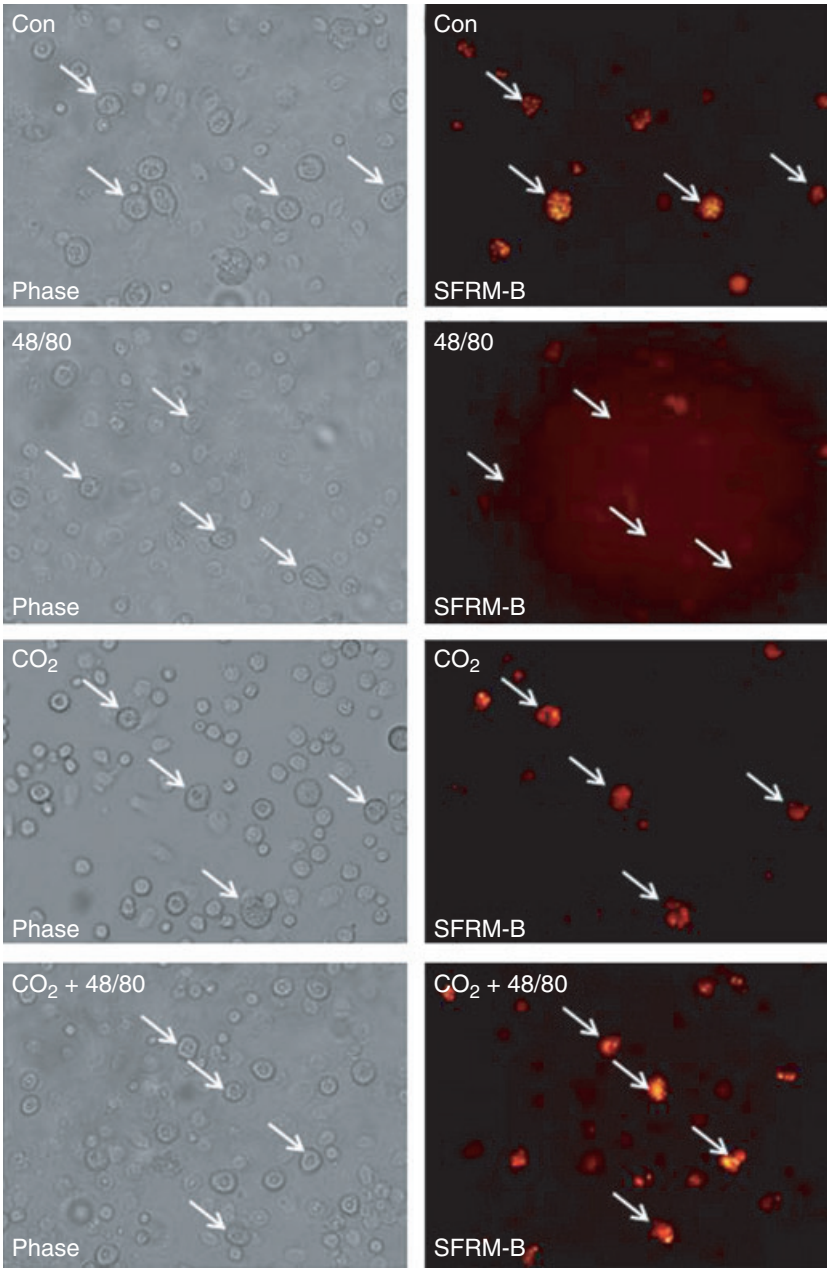

Figure 3 Effects of CO_2 on induced mast cell degranulation. Mast cells loaded with the fluorescent dye SFRM-B were left untreated (control, CON), incubated with compound 48/80 for 15 min (48/80), exposed to 100% CO_2 for 5 min then incubated for 15 min (CO_2), or pretreated with 100% CO_2 for 5 min and then incubated with compound 48/80 for 15 min (CO_2 + 48/80). For each condition, mast cells were visualized under both phase (left panels) and fluorescent microscopy (right panels) to observe the effect of CO_2 on induced mast cell degranulation. Phase pictures were taken prior to 15- min treatment with compound 48/80. Arrows indicate examples of SFRM-B positive mast cells from each condition.

Effects of CO_2 on 48/80-induced histamine release

As a complementary approach to confirm CO_2 is preventing mast cell degranulation, levels of extracellular histamine were determined by EIA in media collected from untreated, stimulated and CO_2 treated cells mast cells. The control untreated condition was used to establish a basal level of histamine in the extracellular media (1.32 ± 0.149 μM). As seen in Fig. 5, when cells were treated with 48/80 (1 μg/ml) for 15 min, there was a significant increase (~4 fold; 5.91 ± 0.834 μM) ($P < 0.0001$) in extracellular histamine concentrations compared to control. Importantly, when mast cells

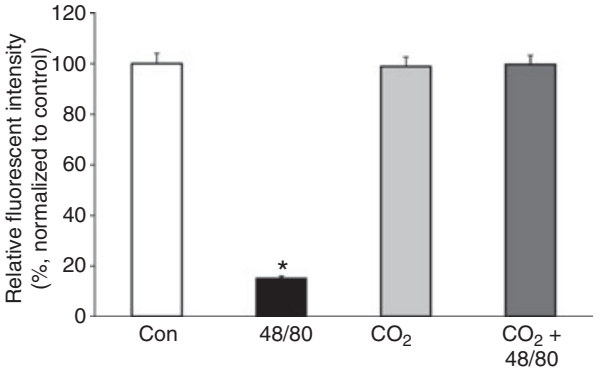

Figure 4 Intensity measurements of mast cell fluorescence. The relative fluorescent intensity levels in mast cells loaded with the fluorescent dye sulforhodamine-B and left untreated (control, CON), incubated with compound 48/80 for 15 min (48/80), exposed to 100% CO_2 for 5 min then incubated for 15 min (CO_2), or exposed to 100% CO_2 for 5 min and then incubated with compound 48/80 for 15 min (CO_2 + 48/80) are shown. Data are presented as the change in relative intensity value when compared to control levels, whose mean was set to equal 100%. *$P < 0.001$ when compared to control levels.

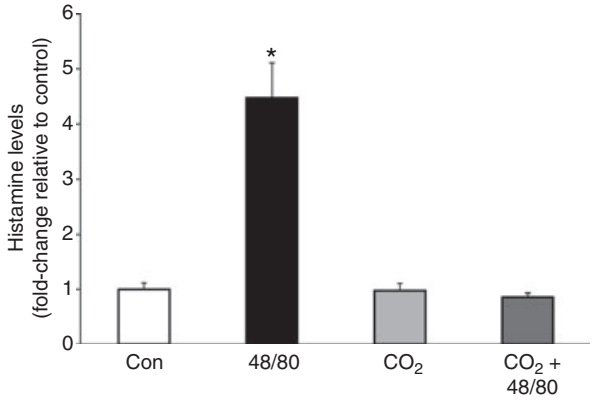

Figure 5 Effects of CO_2 on 48/80-induced histamine release. Mast cells were left untreated (control, CON), incubated with compound 48/80 for 15 min (48/80), exposed to 100% CO_2 for 5 min then incubated for 15 min (CO_2), or pretreated with 100% CO_2 for 5 min and then incubated with compound 48/80 for 15 min (CO_2 + 48/80). Histamine levels were determined by enzyme immunoassay, and data are presented as fold change of histamine concentration when compared to control levels (1.32 ± 0.149 μm). *$P < 0.0001$ when compared to control levels.

were pretreated for 5 min with 100% CO_2, then exposed to 48/80 (1 μg/ml) for 15 min, there was no significant change in histamine concentration (1.12 ± 0.098 μM) when compared to control levels. Furthermore, pretreatment of mast cells with 100% CO_2 for 5 min did not result in a significant change in histamine levels (1.29 ± 0.175 μM). Thus, these data provide additional evidence that CO_2 treatment significantly represses stimulated mast cell degranulation.

Effects of CO_2 on 48/80-induced elevation of intracellular calcium levels

To determine whether the inhibitory effect of CO_2 might involve blocking increases in intracellular calcium levels, cells were labelled with the calcium-binding dye Fura-2 and changes in calcium levels measured in untreated and treated cells. As seen in a representative plot, the addition of compound 48/80 caused a rapid transient increase in intracellular calcium levels to a value much greater than the basal calcium level (Fig. 6A). However, pretreatment of cultures with 100% CO_2 for 5 min greatly repressed the magnitude of the intracellular calcium rise in response to compound 48/80 treatment (Fig. 6B). The mean and standard error were determined for each independent experiment ($N = 3$) and presented in a graph to illustrate the changes in calcium levels among conditions, which are reported as change in peak amplitude over basal levels (Fig. 6C). The average relative calcium increase over basal was 0.68 ± 0.03 ($N = 38$) for mast cells treated with 48/80 but was significantly lower for mast cells treated with CO_2 0.21 ± 0.06 ($N = 26$).

Discussion

Data from our study provide evidence of a novel mechanism that may help to explain the effectiveness of noninhaled intranasal delivery of 100% CO_2 to treat allergic rhinitis by inhibiting mast cell degranulation and histamine release. In the clinical study of Casale et al., (7) intranasal delivery of CO_2 resulted in a rapid and sustained relief of seasonal allergic rhinitis symptoms when compared to placebo-treated response. The study met its primary endpoint, which was a reduction in total nasal symptom score based on the subject's evaluation of the intensity of four nasal symptoms that included congestion, rhinorrhea, itching and sneezing. On the basis of our results, the use of CO_2 as a treatment that directly prevents mast cell degranulation differs from most other commonly used therapies for treating allergic rhinitis that block inflammatory events downstream of mast cell degranulation (6). Allergen and neuropeptide-induced degranulation of mast cells results in the release of histamine as well as other molecules that initiate and maintain the inflammatory response (8, 9, 15, 17, 18, 20). Thus, CO_2 suppression of induced mast cell degranulation can prevent release of inflammatory mediators, such as histamine, which are known to play key roles in the underlying pathology of allergic rhinitis.

In our study, we found that treatment of isolated peritoneal mast cells with the secretagogue 48/80 caused rapid degranulation. The isolation and experimental procedure used in our study was similar to the method described by Wang et al. (29). As expected, our results were in agreement with their findings that demonstrated that 48/80 could cause mast cell degranulation. Mast cells have the ability to undergo degranulation because of immunological and nonimmunological stimuli. We chose to use the nonimmunological secretagogue compound 48/80 to activate and degranulate mast cells since previous studies reported that 48/80 can preferentially activate rat peritoneal mast cells to endocytose

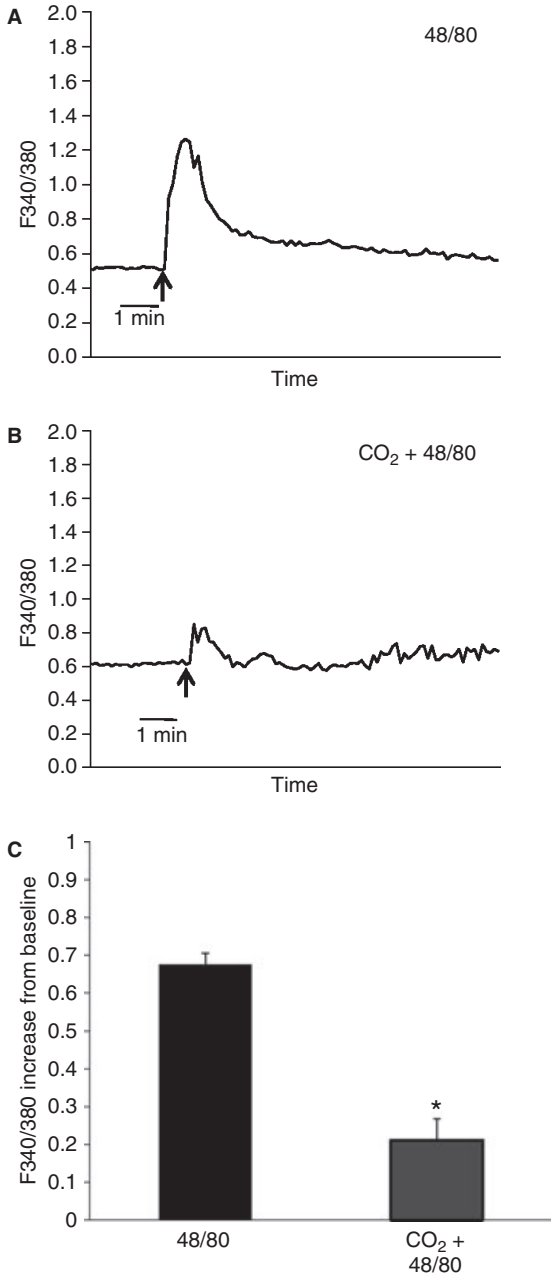

Figure 6 Effects of CO_2 on 48/80-mediated elevation of intracellular calcium levels. Representative plots of 340/380 ratio changes recorded from rat peritoneal mast cells incubated in IH HBS in response to 1 μg/ml 48/80 exposure (A) or pretreatment with 100% CO_2 for 5 min before the addition of 48/80 (B). For each condition, a baseline calcium level was taken for 2 min before the addition of compound 48/80. Arrows indicate time point at which 48/80 was added. The average change in the 340/380 ratio from the baseline value to peak value ± SEM in response to each treatment is shown in panel C. *$P < 0.01$ when compared to baseline levels alone.

compounds in the extracellular medium in a concentration dependent manner (29, 32, 33). This property allows for labelling of mast cells with the fluorescent dye SFRM-B at a lower concentration and release of granules containing the dye from mast cells into the extracellular medium at higher concentrations of 48/80. Although compound 48/80 does not mimic IgE mediated degranulation, it does mimic neuropeptide receptor-mediated degranulation through binding mast cell G-protein couple receptors and activation of the PLC/IP3/DAG pathways leading to an increase in intracellular calcium and thus histamine release (18, 20, 34–36). Regardless of which receptor is being activated, both IgE-mediated and 48/80-mediated degranulation result in elevated intracellular calcium levels. Importantly, we found that pretreating mast cells with 100% CO_2 could significantly suppress 48/80-induced degranulation. To our knowledge, this is the first evidence that treatment of mast cells with CO_2 is sufficient to block degranulation.

Given that both IgE- and 48/80-mediated mast cell degranulation involve increases in intracellular calcium (37, 38) and we had previously reported CO_2 treatment of trigeminal neurons inhibits intracellular calcium increases (28), we wanted to determine whether CO_2 suppression of mast cell degranulation might also involve repression of stimulated calcium levels. It is well established that increases in intracellular calcium levels is required for mast cell degranulation by facilitating the recruitment of vesicle docking proteins to the cell membrane (26, 27). In this study, we found when mast cells were challenged with a degranulating concentration of compound 48/80, there was a sharp transient rise in intracellular calcium that temporally correlated with exocytosis of mast cell vesicles. However, when cells were pretreated with 100% CO_2 for 5 min, the rise in intracellular calcium in response to 48/80 was significantly reduced. On the basis of our findings, we propose that CO_2 inhibition of mast cell degranulation involves repressing the initial increase in intracellular calcium levels that is required for vesicle docking and release.

Our results from this study extend our knowledge about how intranasal CO_2 administration might function at the cellular level to treat allergic rhinitis. In our previous study, CO_2 treatment was shown to block calcium-mediated secretion of the inflammatory neuropeptide calcitonin gene-related peptide (CGRP) from cultured trigeminal ganglion neurons (28). CGRP, which is released in the nasal mucosa in response to trigeminal nerve activation, is implicated in the pathology of allergic rhinitis by promoting vasodilation and glandular secretions, thus contributing to congestion. Trigeminal nerves express receptors for many inflammatory molecules such as histamine (3), cytokines (39, 40) and chemokines (40, 41) that are released from activated mast cells. Therefore, we can speculate that during attacks of allergic rhinitis, mast cell degranulation and subsequent release of inflammatory mediators activate trigeminal nerves and facilitate the release of CGRP, substance P and other molecules that sustain an inflammatory response. These events would establish an inflammatory loop at the level of the nasal mucosa that contributes to the pathology associated with allergic rhinitis. In addition, trigeminal nerve activation also is likely to contribute to other symptoms of allergic rhinitis including sinus pain, itching and sneezing.

On the basis of our findings, we propose that intranasal CO_2 treatment could exert a dual therapeutic benefit by suppressing mast cell degranulation and trigeminal nerve activation.

In summary, results from our study provide evidence of a novel mechanism by which treatment with 100% CO_2 inhibits mast cell degranulation. Furthermore, based on our findings, we propose that the clinical benefit reported for noninhaled intranasal delivery of 100% CO_2 to treat allergic rhinitis could involve inhibition of mast cell degranulation as well as trigeminal nerve activation, two key events implicated in the underlying pathology of allergic rhinitis.

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