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Single-Cell Analysis of Mast Cell Degranulation Induced by Airway Smooth Muscle-Secreted Chemokines

Benjamin M. Manning^{a,1}, Audrey F. Meyer^{a,2}, Sarah M. Gruba^a, and Christy L. Haynes^{a,*}

^aDepartment of Chemistry, University of Minnesota, Minneapolis, Minnesota, 55455, United States

Abstract

Background—Asthma is a chronic inflammatory disease characterized by narrowed airways, bronchial hyper-responsiveness, mucus hyper-secretion, and airway remodeling. Mast cell (MC) infiltration into airway smooth muscle (ASM) is a defining feature of asthma, and ASM regulates the inflammatory response by secreting chemokines, including CXCL10 and CCL5. Single cell analysis offers a unique approach to study specific cellular signaling interactions within large and complex signaling networks such as the inflammatory microenvironment in asthma.

Methods—Carbon fiber microelectrode amperometry was used to study the effects of ASM-secreted chemokines on mouse peritoneal MC degranulation.

Results—MC degranulation in response to CXCL10 and CCL5 was monitored at the single cell level. Relative to IgE-mediated degranulation, CXCL10- and CCL5-stimulated MCs released a decreased amount of serotonin per granule with fewer release events per cell. Decreased serotonin released per granule was correlated with increased spike half-width and rise-time values.

Conclusions—MCs are directly activated with ASM-associated chemokines. CXCL10 and CCL5 induce less robust MC degranulation compared to IgE- and A23187-stimulation. The kinetics of MC degranulation are signaling pathway-dependent, suggesting a biophysical mechanism of regulated degranulation that incorporates control over granule trafficking, transport, and docking machinery.

General Significance—The biophysical mechanisms, including variations in number of exocytotic release events, serotonin released per granule, and the membrane kinetics of exocytosis that underlie MC degranulation in response to CXCL10 and CCL5 were characterized at the single cell level. These findings clarify the function of ASM-derived chemokines as instigators of MC degranulation relative to classical mechanisms of MC stimulation.

*Corresponding author: chaynes@umn.edu.

¹Current Address: University of Minnesota Medical School, Minneapolis, Minnesota, 55455, United States

²Current Address: Intel Corporation, 2501 NW 229th Ave, Hillsboro OR 97124

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Keywords

Carbon-fiber microelectrode amperometry; exocytosis; asthma

1. INTRODUCTION

Affecting 22 million people in the United States alone, asthma is a common chronic respiratory disease.[1] However, relatively little is known about the pathophysiological mechanisms by which it develops and progresses. Additionally, although the use of inhaled glucocorticoid steroids is effective for many patients, a significant percentage respond poorly to these drugs.[1–4] Understanding the pathophysiology of asthma is essential for the development of novel therapies and treatments.

Traditionally, eosinophils have been considered the central orchestrators of asthmatic inflammation. However, recent advances have called this paradigm into question.[2–6] Most notably, a cohort of asthmatic patients exhibits airway inflammation that lacks eosinophilic infiltration. A thorough comparison between asthma and eosinophilic bronchitis, a common and reversible inflammatory disease of the airway, suggests that mast cells (MCs) rather than eosinophils are critical to asthma development.[3,7] MCs are tissue-resident leukocytes derived from hematopoietic progenitors. Mature MCs are identified by the expression of c-kit and the high affinity immunoglobulin E (IgE) receptor (FcεRI), as well as the large number of dense-body granules that occupy the MC cytoplasm. The dense body granules contain many immunoactive mediators, including tumor necrosis factor alpha (TNFα), histamine, serotonin, tryptase, and a sulfated proteoglycan matrix, which are released upon stimulation via exocytosis. MCs also secrete many other species, including cytokines and arachidonic acid derivatives, that are synthesized de novo upon activation.[4,6,8,9] Therefore, MCs can broadly influence the immune system in response to a diverse set of environmental signals. Although their role in allergy has been well studied, MC participation in non-allergic inflammatory diseases is less clearly defined.[4–6,10,11] In addition to IgE-mediated activation, MCs selectively respond to a long list of non-allergic stimuli, including but not limited to, bacterial lipopolysaccharides, neuropeptides, and complement components.[3,4,6,8,9,12] In asthma, MCs are thought to play an important role in displayed symptoms due to their elevated density and activated phenotype in the asthmatic lung. They release proinflammatory factors that promote the Th2-type of inflammation observed in asthma.[5,12–15] Furthermore, many cases of asthma are atopic and present with elevated levels of serum IgE, arguing for the importance of MC degranulation in these patients.[3,4,12] Microlocalization of MCs into the ASM bundles is likely regulated through chemokines secreted by ASM cells in the asthmatic lung. Specifically, CXCL10 and CCL5 have been implicated in the recruitment of MCs via the chemokine receptors CXCR3 and both CCR1 and CCR3, respectively.[14–17] The activity of CXCL10 and CCL5 on MC function has largely been considered chemotactic in nature. Some limited evidence has suggested these chemokines may additionally promote partial degranulation behavior in MCs.[18,19] If significant, CXCL10- and CCL5-induced MC degranulation may, in turn, contribute to the structural remodeling and hypersensitive ASM phenotype observed in asthma.[5,20,21]

To characterize the effects of CXCL10 and CCL5 on mouse peritoneal MC (MPMC) degranulation, carbon-fiber microelectrode amperometry (CFMA) was used, offering real time, direct analysis of CXCL10- and CCL5-induced MC degranulation at the single cell level. Carbon-fiber microelectrodes have high sensitivity and a low, stable background that facilitates single-cell analysis of MC degranulation.[22–27] During CFMA measurements, a microelectrode is set to an oxidizing potential and placed in contact with a single MC. A micropipette provides local delivery of a stimulating substance used to induce MC degranulation. Upon stimulation, cytoplasmic granules release their contents via exocytosis, serotonin is subsequently oxidized at the electrode surface, and the resulting current is monitored over time. The sensitive and dynamic detection of serotonin from single cytoplasmic granules provides a useful handle to monitor the release of the many immunoactive mediators in response to various stimuli.

The data from each CFMA measurement is manifested as a series of individual current ‘spikes,’ each corresponding to the contents of a single MC granule released to the extracellular space (Fig. 1). Characterization of individual current spikes allows quantification of MC secretory function.[25,26,28] In this study, five key parameters were monitored to quantify mast cell secretory function: total serotonin per MC, spike area (Q), spike frequency, spike half-width ($t_{1/2}$), and spike rise time (t_{rise}). Total serotonin, calculated using the number of spikes per cell and the average amount of serotonin released per granule, reflects the overall degree of intragranular contents released per MC. Spike area, the integration of each current spike over time, relates to the number of serotonin molecules released per granule, as determined using Faraday’s law. Spike frequency is measured as the number of release events detected over the course of degranulation and reflects the overall efficiency of the granule trafficking, docking, and fusion processes of exocytosis. Both spike half-width and spike rise-time measurements relate to the kinetics of exocytosis. Spike half-width reflects the overall rate of intragranular content release, and encompasses the combined effects of several terminal stages of exocytosis including fusion pore and intragranular matrix expansion. Spike rise-time relates specifically to the kinetics of fusion pore expansion following fusion of the granular and cell membranes. Together, spike half-width and spike-rise time provide insight into the mechanisms by which the release of MC granule contents is regulated. These parameters obtained from CFMA measurements allow changes in MC secretory function to be described in concrete terms and are useful for the characterization of processes that regulate MC degranulation within the context of asthma.

Our findings provide confirmation, through single cell analysis, that both CXCL10 and CCL5 directly induce degranulation from MPMCs. Our single-cell approach provides a unique and detailed description of chemokine-induced MC degranulation relative to traditional IgE-or calcium ionophore-mediated MC activation. Importantly, using CFMA analysis we describe a consistent and uniform biophysical mechanism by which MC degranulation is differentially modulated in a stimulation-dependent manner.

2. MATERIALS AND METHODS

2.1 Primary mast cell isolation and co-culture

MPMCs were obtained from age-matched, sex-matched C57/BL6 mice (Jackson Laboratories) following IACUC approved guidelines (Protocol # 0806A37663). MCs were isolated by peritoneal lavage and cultured as described previously.[23,26] The peritoneal cavity was injected with 8–10 ml of DMEM high glucose media supplemented with 10% bovine calf serum and 1% penicillin/streptomycin (all from ThermoFisher Scientific, Pittsburgh, PA). Extracted lavage fluid was stored on ice for transport and the collected cells were washed via centrifugation at 400 x g for 7 minutes followed by re-suspension in warm, fresh cell culture media containing either 0.5 $\mu\text{g ml}^{-1}$ anti-TNP IgE (BD Biosciences, San Jose, CA), or vehicle for control conditions. The resuspended cells were then plated onto 35 $\times$ 10 mm Petri dishes containing a confluent layer of murine 3T3 fibroblasts purchased from American Type Cell Culture (Manassas, VA) to create a co-culture environment and incubated overnight. Immediately before use in CFMA experiments, the cell culture media was removed and the cells were washed three times with warm Tris buffer (12.5 mM tris(hydroxymethyl)aminomethane hydrochloride, 150 mM NaCl, 4.2 mM KCl, 5.6 mM glucose, 1.5 mM CaCl_2 , 1.4 mM MgCl_2 , sterile filtered and pH balanced to 7.2–7.4).

2.2 Fabrication of carbon-fiber microelectrodes and glass micropipettes

Carbon-fiber microfiber electrodes were fabricated in lab following a procedure adapted from the method reported by Wightman et al.[23,29] In short, for the production of two microelectrodes, a single carbon fiber (Amoco, Greenville, SC) is aspirated into a glass capillary (A-M Systems, Carlsborg, WA). The capillary, with the carbon fiber within, is then separated into two halves using a pipette puller (Narishige International, East Meadow, NY), each of which consists of a tapered glass tip surrounding the single carbon fiber. The fiber connecting the two halves of the capillary is then cut, separating the two electrodes. Under a microscope, the portion of the carbon fiber extending beyond the insulating glass was then trimmed with a surgical blade. The electrodes were sealed with epoxy (Epoxy Technology, Billerica, MA) and cured at 100 °C for 24 h. Prior to use in carbon-fiber microelectrode amperometry experiments, electrodes were beveled to a 45° angle using a diamond-coated polishing wheel and stored in isopropyl alcohol. Glass micropipettes were also fabricated in lab using a similar procedure. Empty glass capillaries were pulled into two halves, each of which were trimmed to produce a pipette with an opening of 10–50 μm in diameter, suitable for delivery of a small amount of stimulating substance to an individual mast cell.

2.3 Carbon-fiber microelectrode amperometry experiments

Carbon-fiber microelectrode amperometry (CFMA) experiments were conducted as previously described.[25,26,29] Briefly, cultured MCs (with or without anti-TNP IgE pre-incubation) were mounted on a temperature controlled Petri dish holder (Warner Instruments, Hamden, CT) and positioned on the stage of an inverted microscope (Nikon, Tokyo, Japan). Prior to measurement, a carbon-fiber microelectrode connected to a headstage and a pulled glass micropipette loaded with a stimulating substance were mounted on Burleigh PCS-5000 piezoelectric micromanipulators (Olympus America, Center Valley, PA) to permit fine positional control in three dimensions. The electrical potential of the

microelectrode was controlled by an Axopatch 200B potentiostat (Molecular Devices, Sunnyvale, CA) and Tar Heel CV software (National Instruments, Austin, TX), written by Michael L.A.V. Heien, was used to control computer interface settings and record data for each amperometric trace.

For each measurement, the carbon-fiber microelectrode was lowered onto the surface of a single mast cell and set to +700 mV versus a silver/silver chloride (Ag/AgCl) reference electrode (Manufacturer info). The electrode potential was selected based on an established procedure for detection of serotonin from MPMCs without interference from histamine or other electroactive species.[30] Upon collection of data, a single bolus of stimulating substance, 10 μ M A23187 (Sigma Aldrich, St. Louis, MO) or 200 ng ml⁻¹ of either TNP-modified ovalbumin (TNP-OVA (ThermoFisher Scientific, Pittsburgh, PA), CXCL10 (Shenandoah Biotechnology, Warwick, PA), or CCL5 (Shenandoah Biotechnology) in Tris buffer, was delivered locally to the cell. CXCL10 and CCL5 concentrations were selected based on physiologically relevant concentrations reported in the literature as well as preliminary bulk assays conducted in the Haynes laboratory.[31,32] Serotonin released to the electrode surface was detected as current over the course of a 90 s collection period.

2.4 Data analysis and statistics

Each amperometric trace obtained from a single CFMA experiment (and thus, representing the degranulation behavior of a single MC) was filtered at 200 Hz through a low-pass Bessel filter and exported as a text file prior to conversion, using the ABF Utility provided in the MiniAnalysis software package (Synptosoft, Fort Lee, NJ), to the .ABF file format required for spike parameter analysis. Using the MiniAnalysis software, each trace was analyzed for the spike parameters discussed above, and an average value for each cell was obtained. Average parameter values for each trace were pooled across similar experimental conditions. Outliers were defined as any trace for which the experimental values were found to be more than two log standard deviations from the log mean for that condition. For example, if a given trace produced all but one parameter within the criteria, the trace would be discarded as an outlier for all measured parameters. Following the removal of outliers, the mean spike parameter values for each trace were averaged and compared to other experimental conditions using the two-tailed Student's t test with statistical significance determined at the 95 percent confidence interval ($p < 0.05$). Comparison of stimulation conditions was carried out in three separate experiments, each with TNP-OVA-stimulated MCs as a control condition; the absolute values used to derive the %TNP-ova data displayed in figures 2–4 can be found in the supporting information. The error bars for the TNP-OVA condition on all graphs are represented as a standard error measurement calculated using a weighted average of the variances the three separate TNP-OVA data sets.

3. RESULTS AND DISCUSSION

Preliminary investigation of bulk MC secretory function was examined in response to various ASM-associated inflammatory mediators using high performance liquid chromatography equipped with electrochemical detection (data not shown). Surprisingly, these experiments, suggested that CXCL10 and CCL5 directly induced MC degranulation in a manner that was independent from IgE-mediated stimulation. The observed effect,

although small relative to IgE-mediated stimulation, warranted further investigation to describe the biophysical processes that underlie these alternate pathways of MC degranulation in greater detail provided by single cell analytical techniques.

To explore the direct induction of MC degranulation by ASM products, CFMA was used to monitor real time MC secretory function at the single cell level. The degranulation behavior of individual mouse peritoneal MCs cultured in vitro and incubated with anti-trinitrophenol (anti-TNP) IgE was monitored using CFMA following direct local exposure to either CXCL10 or CCL5, as well as both TNP-modified ovalbumin (TNP-OVA), as a model for IgE-mediated degranulation, and the calcium ionophore A23187 as separate positive controls. An additional experimental condition consisted of A23187-stimulated MCs without IgE pre-incubation. In contrast to IgE-mediated signaling, A23187 directly shuttles calcium ions across the cell membrane to induce degranulation.

The results of these experiments clearly demonstrate the ability of both CXCL10 and CCL5 to directly induce MC degranulation and highlight the distinct differences in degranulation behavior between alternative modes of stimulation. Fig. 1 shows representative CFMA traces recorded from each experimental condition. Calculation of total serotonin released per cell reveals that CXCL10 directly induces MC degranulation; however, CXCL10-stimulated MCs released 52% less serotonin per cell than the IgE-mediated condition (Fig. 2). CCL5 also directly induced MC degranulation to a lesser degree, equal to 89% less serotonin than the IgE-mediated condition (Fig. 2). In contrast to the CXCL10 and CCL5 stimulation, calcium ionophore-induced degranulation (A23187) was more robust than the IgE-mediated (TNP-OVA) MC response. A23187, both with and without IgE incubation, inducing stronger MC degranulation responses, releasing 62% and 27% more serotonin per cell, respectively, than the TNP-OVA stimulation (Fig. 2). However, only the A23187-stimulated MCs pre-incubated with anti-TNP IgE was found to be significantly different from TNP-OVA-stimulated counterparts. This data confirms the direct MC degranulation activities of both CXCL10 and CCL5, and highlight the ability of CFMA to directly compare stimulatory conditions and reveal distinct stimulation-dependent MC degranulation patterns at the single cell level. Additionally, these findings suggest a dual role of ASM-secreted products in asthma pathogenesis. The role of CCL5 and CXCL10 is not strictly important for the microlocalization of MCs to the ASM, but these cytokines directly participate in the MC activation and degranulation to influence the proinflammatory ASM phenotype in asthma. This work therefore supports the emphasis on IgE-independent modes of mast cell activation, particularly via ASM-secreted products, as promising pathways to target for the development of novel asthma therapies.

In addition to demonstrating the ability of CXCL10 and CCL5 to directly induce MC degranulation, CFMA facilitated the characterization of MC secretory function by monitoring individual cells in real time and with precise control of stimulatory conditions. In particular, our group used CFMA to characterize the partial degranulation observed in MPMCs stimulated via CXCL10 or CCL5 relative to IgE- and A23187-mediated MC degranulation. The results presented here clearly demonstrate a wide range of MC degranulation (as measured by the degree of serotonin released) that is highly stimulation-

dependent. Individual spike analysis was conducted to investigate the biophysical mechanism by which MC degranulation is differentially regulated.

Across all conditions, the serotonin released per granule, as measured by spike area values, corresponded directly to the amount of total serotonin released per cell. A23187 stimulated MCs pre-treated with anti-TNP IgE released 150% of the serotonin released per granule observed in the TNP-OVA condition; slightly more than the 145% of TNP-OVA observed from MCs stimulated with A23187 without IgE incubation (Fig. 3a). Conversely, CXCL10- and CCL5-stimulated MCs released only 75% and 60%, respectively, of TNP-OVA-stimulated MCs (Fig. 3a). All observed changes were statistically significant ($p < 0.05$). Because all incubation conditions were identical (with the exception of A23187-stimulated MCs without IgE pre-treatment), it is unlikely the observed stimulation-dependent changes in serotonin released per granule are due to altered granule loading. Rather, our results suggest CXCL10- and CCL5-induced partial degranulation in MCs is a regulated process and involves modulated and fractional release of granular contents.

The contributions of altered spike area to the total amount of serotonin released per cell were amplified by changes in measured spike frequency. A23187-stimulated MCs, both with and without IgE incubation, were found to undergo degranulation at measured frequency values 34% and 16% greater than TNP-OVA-stimulated cells, respectively, though the latter was not statistically significant ($p = 0.09$) (Fig. 3b). In contrast, stimulation with CXCL10 and CCL5 produced degranulation frequency values 48% and 75% lower, respectively, than the TNP-OVA condition (Fig. 3b). These findings are clear evidence that granule trafficking and docking efficiency are also regulated in a stimulation-dependent manner that influences the degree of MC degranulation.

Therefore, our findings argue that, compared to IgE-mediated degranulation, the relatively lower total serotonin released from MCs directly stimulated by CXCL10 and CCL5 results from both decreased serotonin released per granule and decreased frequency of release events. Similarly, the relatively larger amount of serotonin released from A23187-stimulated MCs with IgE is due to greater amounts of both serotonin released per granule and increased granule release frequency. Interestingly, the A23187-stimulated MCs without IgE pre-incubation did not demonstrate significantly increased levels of total serotonin compared to TNP-OVA, even though increased serotonin per granule was observed. This discrepancy can be attributed to the lack of a significantly elevated granule release frequency in the A23187-stimulated cells without IgE preincubation. This implies surface-bound IgE may influence MC degranulation by removing barriers to granule trafficking and docking efficiency. Overall, this data suggests a unified biophysical mechanism by which MCs exhibit pathway-dependent modulation of degranulation intensity. Most importantly, MC degranulation induced by CCL5 and CXCL10 are distinct from IgE-mediated activation. These findings highlight the variations in MC degranulation that demand consideration when evaluating the role of MCs in asthma.

Given the role of both spike area (loaded serotonin) and spike frequency (granule transport, docking, and fusion) in the regulation of MC degranulation, further spike analysis was conducted to gain more insight into this process. Both spike half-width and spike rise-time

were monitored as indicators of the intragranular matrix and membrane driving forces that are important to the exocytosis process. Consistent trends were observed for these parameters across all monitored conditions. As spike area decreased, both spike half-width and spike rise-time increased significantly (Fig. 4a,b).

For example, considering the observed 50% increase in spike area measured for A23187-stimulated MCs with anti-TNP IgE incubation versus TNP-OVA, the same condition demonstrated a 22% decrease in spike half-width (Fig. 4a). Similarly, A23187-stimulated MCs without IgE incubation showed a 45% increase in spike area and a 24% decrease in spike half-width compared to the TNP-OVA-stimulated condition (Fig. 4a). Following the same trend, the CXCL10- and CCL5-stimulated MCs, which released 25% and 40% less serotonin per granule versus TNP-OVA, responded with increased spike half-widths of 35% and 50% compared to IgE-mediated degranulation, respectively (Fig. 4a). These findings strongly support an important role for membrane driving forces in regulating the degree of granular content release, perhaps in conjunction with changes in intracellular signaling, including the Ca^{2+} signaling cascade.[33,34] In the absence of altered membrane dynamics, larger spike area values may correspond to increases in spike half-width due to increased time to release more granular cargo. Importantly, the opposite trends observed in this work (decreased spike area associated with increased half-width) suggest membrane driving forces directly participate in the regulation of MC degranulation.

Additionally, A23187-stimulated MCs with and without IgE demonstrated spike rise-time values 19% and 23% less, respectively, compared to TNP-OVA values despite the observed increases in serotonin released per granule (Fig. 4b). In contrast, CXCL10- and CCL5-stimulated MCs demonstrated increased spike rise-time values 18% and 52% greater, respectively, than TNP-OVA-stimulated MCs suggesting intragranular matrix effects and/or altered fusion pore stability may also participate in the regulatory mechanism of MC degranulation (Fig. 4b). As with spike half-width, a decrease in spike area combined with a corresponding increase in spike rise-time is consistent with the direct involvement of intragranular matrix effects and/or altered fusion pore formation characteristics in the stimulation-dependent regulation of MC degranulation.

3. CONCLUSIONS

In summary, the research presented herein demonstrates the utility of CFMA to permit direct observation, in real time, of MC degranulation behavior at the single cell level, and specifically for the study of asthma-relevant ASM products on MC function. Notably, these findings confirm the capacity of the ASM-secreted chemokines CXCL10 and CCL5 for the direct induction of MC degranulation at total secretion levels significantly lower than conventional MC degranulation pathways such as IgE- and A23187-mediated stimulation. The dual activity of CXCL10 and CCL5 on MC function, although indicative of a more complex ASM-MC interaction, suggests a simple model of MC microlocalization to the ASM and subsequent activation in asthma, presumably upon exposure to a threshold cytokine concentration. Importantly, this work reveals consistent trends in the biophysical mechanism of regulated MC degranulation in response to different stimuli. Across all five experimental conditions, increases in the extent of MC degranulation (both A23187-

stimulated conditions relative to the IgE pathway) were accomplished by a combination of increased serotonin released per granule and an increased frequency of release events. Conversely, a decreased degree of degranulation (CXCL10- and CCL5-stimulated conditions relative to the IgE pathway) were accounted for by both decreased serotonin released per granule and a decreased frequency of release events. Furthermore, observed trends in spike half-width and rise-time parameters, both increasing with corresponding decreases in spike area, also suggest a consistent role of intragranular matrix and membrane driving forces in the stimulation-dependent release of granular contents. In the absence of granule loading effects (similar incubation and cell culture conditions), these findings argue for a unified biophysical mechanism of regulated degranulation that is pathway independent and incorporates control over granule trafficking, transport, and docking machinery as well as intragranular matrix and membrane driving forces. The extent to which this model of regulated degranulation is conserved across all modes of MC stimulation will be the subject of further study. A complete understanding of the relationship between ASM and MCs in the pathogenesis of asthma will require extensive further research. Nonetheless, the findings outlined here emphasize the need for a more nuanced understanding of the multidimensional and complex nature of cell-cell interactions and the inflammatory response in asthma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- CFMA allows direct observation of single cell mast cell degranulation in real time
- Both CXCL10 and RANTES, airway smooth muscle-relevant cytokines, are capable of directly inducing mast cell degranulation
- Mast cells are capable of stimulation pathway-dependent regulation of exocytosis

Figure 1.

Representative CFMA traces from MCs incubated with $0.5 \mu\text{g ml}^{-1}$ anti-TNP (with the exception of the A23187-stimulation without IgE condition) IgE and stimulated via calcium ionophore ($10 \mu\text{M}$ A23187), IgE-mediated activation (200 ng ml^{-1} TNP-OVA), or ASM-secreted cytokines (200 ng/ml CXCL10 or CCL5).

Figure 2.

Total serotonin released from MCs stimulated with A23187 without IgE pre-incubation (n=18), A23187 with IgE pre-incubation (n=17), TNP-OVA (n=19), CXCL10 (n=26), or CCL5 (n=16). Total serotonin is calculated using the average Q value per spike and the number of spikes recorded for a given CFMA trace. * indicates $p < 0.05$ versus TNP-OVA condition. "n" refers to the number of traces that were collected.

Figure 3.

Average spike area (A) and spike frequency (B) values for MCs stimulated with A23187 without IgE pre-incubation (n=18), A23187 with IgE pre-incubation (n=17), TNP-OVA (n=19), CXCL10 (n=26), or CCL5 (n=16). * indicates $p < 0.05$ versus TNP-OVA condition. “n” refers to the number of traces collected.

Figure 4.

Average spike half-width (A) and spike rise-time (B) values for MCs stimulated with A23187 without IgE pre-incubation (n=18), A23187 with IgE pre-incubation (n=17), TNP-OVA (n=19), CXCL10 (n=26), or CCL5 (n=16). * indicates $p < 0.05$ versus TNP-OVA condition. “n” refers to the number of traces collected.