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The Use of Microelectrode Array (MEA) to Study Rat Peritoneal Mast Cell Activation

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Abstract. We performed this study to demonstrate the applicability of the microelectrode array (MEA) to study electrophysiological changes of rat peritoneal mast cells in the presence of compound 48/80 under normal, Ca^{2+}-free, Ca^{2+}-free with EDTA, and Cl^--free conditions. The use of high extracellular K^+ (KCl, 150 mM), charybdotoxin (ChTX, 100 nM), and Cl^--free containing ChTX buffers verified that the hyperpolarizing signal was due to the activation of mainly K^+ and, to a lesser extent, Cl^- channels. Compound 48/80 concentration-dependently shortened the latent periods (the onset of response) and increased both the spatial (the K^+ and Cl^- hyperpolarizing field potentials, HFP) and temporal measurements (the duration of response). Ca^{2+}-free buffer had no effect on the latent period of compound 48/80 but increased the HFP at high concentrations. The latent period increased while the HFP diminished when cells were equilibrated in Ca^{2+}-free buffer containing EDTA. Durations of the HFP were generally longer when cells were in either Ca^{2+}-free or Ca^{2+}-free containing EDTA buffers than when cells were in normal buffer. The EC_{50} values confirmed that effects were only affected in Ca^{2+}-free buffer containing EDTA but not in Ca^{2+}-free or Cl^--free buffers, further reinforcing the hypothesis that the presence of Ca^{2+} is not essential to the action of compound 48/80. The present study is the first application of MEA to study rat peritoneal mast cells, and our results indicate that it could be of value in future pharmacological research on other non-excitable cells.

Keywords: rat peritoneal mast cell, hyperpolarizing field potential, potassium channel, calcium channel, microelectrode array

Introduction

Mast cells are involved in type-1 allergic responses, typified by the exocytotic release of histamine and other mediators following immunological as well as non-immunological challenges (1), and this release of histamine appears to be dependent on cytoplasmic calcium concentration ($[Ca^{2+}]_i$) (2, 3). It has long been considered that extracellular Ca^{2+} influx is important in the mechanism of degranulation (4 – 6), with intracellular Ca^{2+} stores also playing a pivotal role in the initial increase of cytosolic Ca^{2+} (7 – 10).

While Ca^{2+} entry and Ca^{2+} release–activated Ca^{2+} (CRAC) channels are obviously important in mast cell activation, ionic gradients of potassium (K^+) and chloride (Cl^-) are likely to also play important roles in mast cell secretory responses through their influence on membrane potential and thus Ca^{2+} influx. Available evidence has revealed that the activation of the outward K^+ current correlates with the activation of CRAC current in both time and amplitude (11) and that a Ca^{2+}-activated K^+ (K_{Ca}) current enhances the release of mediators (12). Furthermore, in contrast to excitable cells, mast cell membrane hyperpolarization is required to support Ca^{2+} entry (6). Indeed, secretagogues, such as compound 48/80, have been shown to cause a fast

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hyperpolarization and an increase in intracellular Ca^{2+} levels (13). It thus appears that K^+ and Ca^{2+} are intrinsically linked and that K_{Ca} channel activation may even be more critical than Ca^{2+} entry in the control of mediator release. Ion channels and mast cell activation have been reviewed (14, 15).

Exocytosis in mast cells can be stimulated non-immunologically by a variety of chemically heterogeneous secretagogues, and one such agent is a synthetic non-peptide molecule known as compound 48/80 (1). Compound 48/80 has been shown to induce the release of Ca^{2+} from intracellular stores (16) via a receptor-independent mechanism through direct activation of certain heterotrimeric G proteins (17, 18). The effects of such a secretagogue on mast cells have been frequently monitored by quantifying histamine release (19, 20). Since there is an intimate relationship between K^+ and Ca^{2+} levels and mast cell degranulation, many studies have utilized conventional electrophysiological techniques, such as patch-clamp, to investigate their contributions to mast cell activation (6, 21 – 23). Reviews on the use of patch-clamp in the study of secretion and ion channels involvement in mast cells have been provided by Penner and Neher (24) and Hoth et al. (25), respectively.

While the use of patch-clamp has proven to be invaluable for the evaluation of drug action on mast cells, the technique itself is difficult and time-consuming to perform. Furthermore, the recorded electrophysiological data from as many patched cells on a given day may vary substantially. The results can be quite different depending on whether the conventional whole-cell attached voltage-clamp configuration or the perforated-patch technique is used and whether the recording is carried out at room temperature (i.e., 22°C – 26°C), which is the case in most patch-clamp experiments, or at 37°C (26).

In view of the need to measure electrophysiogical changes of mast cells both quickly and efficiently as well as to provide a convenient means of screening a large amount of potential anti-allergic compounds on mast cell activation, we propose the use of microelectrode arrays (MEAs). The concept of the MEA is based on the integration of biological cells on microchips in order to detect changes of extracellular field potentials. Our current devices and amplifier systems have high signal-to-noise ratios (approximately 50 to 100) and are much more reliable than previous versions. The MEA chips are durable and can be reused many times (>50). Also, the present MEA system is sensitive enough to allow the recording of extracellular signal shape changes, which can be used to determine drug potencies and effects (27). At present, MEA systems are mainly being used to study neuronal (28 – 30), cardiac (31, 32), and gastrointestinal functions (33).

Our previous studies have demonstrated the potential application of cardiac myocytes–integrated field-effect transistor arrays in pharmacological bioassay and, in particular, for the interpretation of the recorded signal shapes (27, 34, 35). Subsequent development has led to the fabrication of our current MEA system, which gives an improved signal-to-noise ratio and provides a load-free voltage measurement without distortion of the extracellular recorded signal shapes (36). The present planar metal microelectrodes enable high input impedance recordings and reliable interpretations of the signal shapes corresponding to the relative contributions of Na^+, K^+, and Ca^{2+} ions.

This study attempts to correlate the present findings with established knowledge of peritoneal mast cells under different physiological conditions by comparing effects of compound 48/80 on cells in normal, Ca^{2+}-free, EDTA-containing Ca^{2+}-free, and Cl^--free buffers. In order to characterize the relationship between K^+ current and peritoneal mast cell activation, high extracellular K^+ concentration and charybdotoxin (ChTX) were used to disrupt the electrochemical gradient of K^+ and to block K_{Ca} channels, respectively. We performed this study to demonstrate the applicability of the MEA to study electrophysiological changes, in terms of field potentials, of peritoneal mast cells.

Materials and Methods

Animals

Rat peritoneal mast cell suspensions were obtained from male Sprague-Dawley (SD) rats. The rats were outbreed within the Laboratory Animal Services Centre of the Chinese University of Hong Kong and were housed at approximately 25°C in 12-h light/dark cycles. The Animal Experimentation Ethics Committee, the Chinese University of Hong Kong, approved the experiments and protocols that were used.

Preparations of media

Standard medium: Standard buffer (pH 7.4) contained 137 mM NaCl, 5.6 mM glucose, 10 mM *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulphonic acid (HEPES, 2.7 mM KCl, 0.4 mM NaH_2PO_4, and 10 mM $CaCl_2$.

Calcium-free and chloride-free media: The Ca^{2+}-free buffer was the same as the standard buffer but without $CaCl_2$ (pH 7.4).

The gluconate buffer (Cl^--free) contained 137 mM $NaC_6H_{11}O_7$, 2.7 mM $KC_6H_{11}O_7$, 0.4 mM $NaH_2PO_4 \cdot 2H_2O$, 1.0 mM $Ca(C_6H_{11}O_7)_2$, 10 mM HEPES, and 5.6 mM glucose. This was used in Cl^--free experiments instead

of the standard buffer.

Calcium-free with ethylenediaminetetraacetic acid (EDTA) medium: The peritoneal mast cell suspension was washed by centrifugation (180 × *g* at 4°C for 5 min) in Ca^{2+}-free buffer and subsequently resuspended in the same buffer containing 0.1 mM EDTA before incubation.

Preparations of peritoneal mast cells

SD rats were sacrificed by decapitation, followed by exsanguination under running water. A 25-ml volume of heparin (50 IU/ml)–containing standard buffer was injected into the SD rat intra-peritoneally. The solution-filled peritoneal cavity was then gently lavaged for 2 min. The injected buffer (containing approximately 4% – 5% peritoneal mast cells at this point) was extracted and centrifuged (180 × *g* at 4°C for 5 min). After discarding the supernatant, the cells were resuspended in standard buffer supplemented with bovine serum albumin (1 mg/ml). Rat peritoneal mast cells were isolated and purified by using a Percoll® density gradient. The cell suspension (1 ml) was mixed with 4 ml of 90% Percoll® and 1 ml of bovine serum albumin–supplemented standard buffer was then carefully layered onto the Percoll®-cell mixture. Purification was performed by centrifugation (150 × *g* at 4°C for 25 min), which allowed cell separation and gradient formation simultaneously. Peritoneal mast cells would gather as a single layer at the bottom of the tube, whereas other cells would form a rather compact layer on top of the gradient. The peritoneal mast cell pellet was then collected and washed twice in standard buffer by centrifugation and finally resuspended at the desired cell density. The peritoneal mast cell purity of >98% was confirmed with toluidine blue staining.

Microelectrode arrays

The MEAs were manufactured on glass wafers (Borofloat 33; SCHOTT GLAS, Mainz, Germany) using standard silicon technology. The planar 64-channel gold microelectrode arrays (8 × 8) were designed with a diameter of 20 μm at a pitch of 100 or 200 μm. In order to use the MEA several times, the chip surface was passivated by an oxide–nitride-oxide layer deposited by plasma enhanced chemical vapor deposition consisting of 500 nm SiO_2, 500 nm Si_3N_4, and 100 nm SiO_2. Details of the fabrication and encapsulation processes have been previously described (32, 37, 38).

We used a custom-made 64-channel amplifier system in which microelectrodes were coupled directly to the inputs of high impedance operational amplifiers (OPA 627, input impedance Z_{in} = 14 TΩ, gain: 33×; Burr-Brown, Texas Instruments, Dallas, TX, USA), and it was then coupled to a main amplifier (gain: 33×) providing an overall gain of 1,089×. The high pass performance of the recording system was only dependent on the size, material, surface condition, and cleanliness of the microelectrodes, providing a large bandwidth recording (36). As a result of this ultimate high impedance input (39), the bandwidth (3 dB cut-off) of the recording system was enhanced to 1.7 Hz – 3.7 kHz for planar gold microelectrodes with a diameter of 30 μm. This performance enables reliable recordings of distinct signal shapes of extracellularly recorded field potentials originating from individual cells. Most importantly, recordings can be done with ease, and it is cheap to fabricate planar gold microelectrodes, which can be designed in sizes smaller than the soma of individual cells if necessary. Data were sampled at 10 kHz per channel, with simultaneous acquisition across all channels (PCI 6071E; National Instruments, Hong Kong) using MED64 conductor 3.1 software (Alpha MED Sciences Co., Ltd., Osaka). An extracellular Ag/AgCl electrode, which was set to ground potential, served as a reference electrode. A more detailed description of the data acquisition has been published previously (32, 27, 38).

Chemicals and reagents

Percoll® was purchased from GE Healthcare Bioscience (Uppsala, Sweden) for gradient-purification. Bovine serum albumin, compound 48/80 ($C_{11}H_{15}NO$), EDTA, and all other chemicals of analytical grade were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Experimental procedures

Peritoneal mast cells were resuspended at a cell density of approximately 3×10^6 cells/ml in the corresponding buffers. About 33 μl of cell suspension (about 10^5 cells) was placed onto the recording area of the MEA (Fig. 1a). The chips, about 15 of them from each culture, were then incubated at 37°C balanced with 5% CO_2 for at least 15 min. They were then transferred to the headstage amplifier followed by an addition of 157 μl of the corresponding buffer. In experiments studying the effects of compound 48/80 on peritoneal mast cells, a bolus dose of 10 μl was delivered by a micro-syringe to give a final MEA volume of 200 μl (i.e., 33 μl cell suspension + 157 μl corresponding buffer + 10 μl drug). The final bath concentrations of compound 48/80 (0.001 – 1.0 μg/ml) were chosen as they are comparable to the concentrations used in other studies. The micro-syringe was secured directly on top of the 64 recording electrodes of an MEA by a micromanipulator mounted on top of the headstage amplifier. Once a particular concentration of an agent had been added and the degranulation process had occurred, another cell-

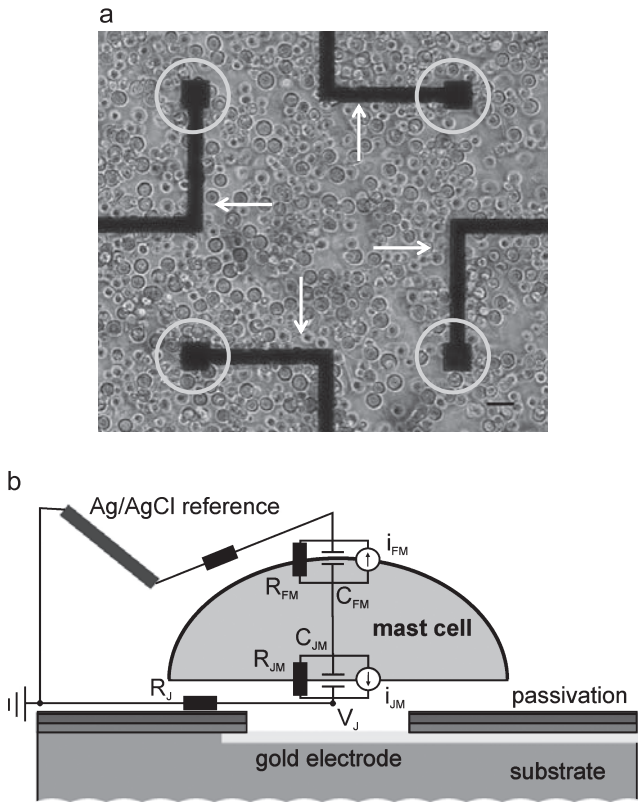

Fig. 1. Mast cells on an MEA and the point contact model for cell signal recording. a: Phase contrast image of peritoneal mast cells seeded on an MEA was taken using an inverted microscope (Scale Bar = 30 μm). Four electrodes (circles) and their connecting lines (arrows) of an MEA are indicated. b: Simplified equivalent electronic contact model for the explanation of the signals recorded from the mast cell with the present system. Ionic currents through the cleft at the junction membrane (JM) over the seal resistance R_J are leading to voltage changes V_J on top of the electrodes, which are then picked up by the MEA.

containing MEA would be used. For the controls, i) 10 μl drug-free standard buffer was added to cell-containing MEAs or ii) 10 μl drug-containing standard buffer was added to cell-free MEAs to ensure that no electrophysiological signals were generated by either the mere injection or the presence of drug.

Signal generation

In general, extracellular signals recorded by MEA chips can be described in a first approximation by the point contact model (40). A schematic of the experimental situation is shown in Fig. 1b. The mast cell is located on the metal electrode and the narrow cleft filled with electrolyte solution is forming a seal resistance R_J. Typical distance values for the cellular membrane from the surface lie in the range of several tens of nm to 100 nm (41), resulting in values of 1 – 10 MΩ for R_J (42). The cellular membrane is separated into a junction membrane (JM) and a free membrane (FM). Electronically the membrane parts can be described by a resistor, capacitor, and current source generating ionic currents over the membrane. The main currents observed in this case are cationic currents such as sodium, potassium, calcium, and anionic currents from chloride. The currents through the attached membrane part i_{JM} need to flow along the seal resistor R_J, leading to a potential drop of V_J at the contact point. The model in general also allows for capacitive currents over C_{JM}. In the present study, as the mast cell signals are much slower than signals usually recorded from neurons or cardiac myocytes, the capacitive components can be excluded here. Inward cationic currents (Na^+ or Ca^{2+}) give rise to signals in a negative direction from the baseline. An inward anionic current (Cl^-) or an outward cationic current (K^+) leads to positive signals from the baseline.

Expression of results and statistics

The results are expressed in terms of i) latent period (s), which is the time from the moment of drug application to the onset of response; ii) spatial (mV); that is, field potential; and iii) temporal (s), which is the time for the field potential recording to return to the baseline; measurements ± S.E.M. As the concentrations used were not linear, the log concentrations are expressed in graphical presentations of the results. Where appropriate, the signal shapes, which comprise all three parameters, are also shown. The above measurements were obtained from an average of at least 7 randomly selected recordings from each MEA chip, which is considered as one experiment (n = 1). The results from high extracellular K^+ (150 mM KCl), normal buffer containing ChTX, and Cl^--free buffer containing ChTX experiments were used to verify that the recorded hyperpolarizing signal shape was the sum of K^+ and Cl^- channel activations. Comparisons of the effect of compound 48/80 on peritoneal mast cells cultured in normal, Ca^{2+}-free, Ca^{2+}-free with EDTA, and Cl^--free buffers are also expressed in terms of EC_{50} (μg/ml ± S.E.M.), that is, the effective concentration that elicited 50% maximal spatial response in the presence of compound 48/80 on a given MEA. As the sensitivity and maximal response to the presence of compound 48/80 varied from culture to culture, the EC_{50} could only be calculated from experiments in which a full concentration-response curve (0.001 – 1 μg/m) was constructed. All results were compared by using 2-way ANOVA followed by the Bonferroni *t*-test or Student's unpaired *t*-test, whichever appropriate. $P<0.05$ was considered to indicate a statistically difference between values.

Results

Activation of mast cells leads to the release of histamine, which can be induced by compound 48/80. This release is preceded by a series of electrophysiological events, and the hyperpolarization induced by the movements of K^+ and Cl^- was being detected by our MEA system in the present study.

To verify the source of recorded field potential

Recording from the present system represents a net gain of intracellular negative charge, either due to an efflux of cations (e.g., K^+) or an influx of anions (e.g., Cl^-), with a positive field potential signal shape. Upon close examination at the signal shapes during the field potential shifts, positive spikes underlying the baseline shifts can be seen. Such signals could either be generated by fast repetitive K^+ efflux or Cl^- influx. In order to definitively classify that the recorded signal shapes were indeed primarily due to an efflux of K^+, medium containing either high K^+ (150 mM) or ChTX (100 nM, 10 min) was used (Fig. 2). In both conditions, the latent periods were not significantly affected, but they were marginally longer when cells were in high K^+ medium (results not shown). The measurable field potentials remained at similar levels regardless of the concentration of compound 48/80 used, and the obtained signal strengths were generally lower in ChTX-treated cells than the results obtained from high K^+ buffer experiments (Figs. 2 and 3a). Interestingly, much higher field potentials were observed at 1.0 μg/ml than at other concentrations. High K^+ buffer increased the temporal periods while ChTX did not (Fig. 3b). Because neither high K^+ nor ChTX completely blocked the cellular response to compound 48/80 at and below 0.3 μg/ml, it suggests that something other than K^+-channel activation was involved in the hyperpolarizing potential observed at these concentrations. Since another major factor that could have caused a net gain in the intracellular negative charge was Cl^- influx, the concentration–response to compound 48/80 was repeated in cells exposed to Cl^--free buffer containing ChTX. In this condition, only very negligible signals could be measured, and the apparent potentials detected at 1.0 μg/ml in high K^+ and ChTX experiments were no longer present, suggesting that compound 48/80 has a significant Cl^- channel–activating action at concentrations close to 1.0 μg/ml (Fig. 2). Although the involvement of Cl^- channels was only

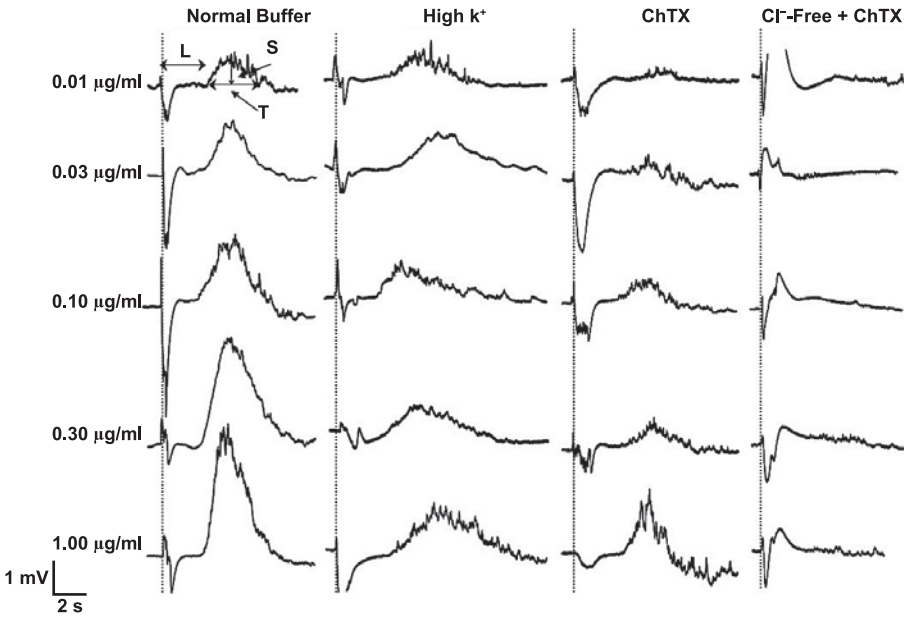

Fig. 2. Field potential recordings of peritoneal mast cells in the presence of compound 48/80 (0.01 – 1 μg/ml) were compared with those obtained when cells were exposed to high extracellular K^+ (150 mM KCl) or charybdotoxin (100 nM ChTX). The latent period (L), spatial (S), and temporal (T) measurements are indicated. The large artefacts along the dotted lines indicate the moment when compound 48/80 was added. Although the presence of high K^+ and ChTX substantially reduced the field potential at concentrations up to 0.3 μg/ml, they did not abolish it completely. It appeared that the temporal measurement was lengthened in high extracellular K^+ condition, but not in the presence of ChTX. Since Cl^- influx is another major hyperpolarizing factor that could have accounted for the net gain in the intracellular negative charge, the effect of compound 48/80 was repeated in the presence of Cl^--free buffer containing ChTX. The results confirmed that the remaining levels were indeed due to Cl^--channel activation as the field potentials found in high K^+ and ChTX conditions at and below 0.3 μg/ml virtually disappeared, and the apparently more substantial potentials detected at 1.0 μg/ml were no longer present.

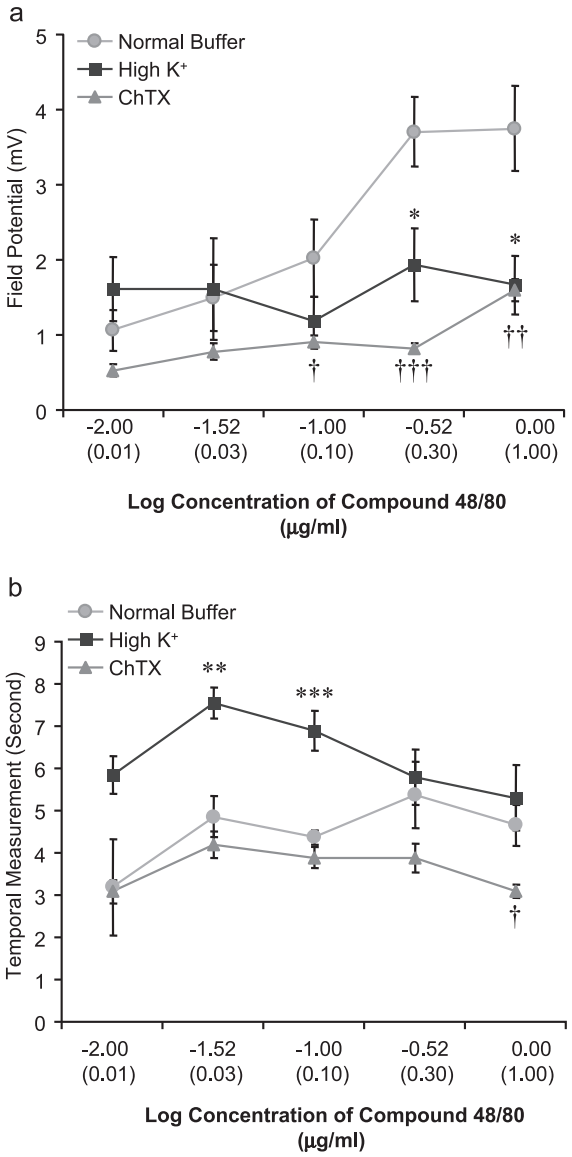

Fig. 3. Effects of compound 48/80 (the log concentration and actual concentrations, μg/ml, in parentheses, n = 3 – 7) on HFP (a) and temporal measurements (b) on peritoneal mast cells cultured in high extracellular K^+– and ChTX-containing buffers. Significant reductions in HFPs were found in cells cultured in either condition with ChTX being more effective than high K^+ at reducing the effect of compound 48/80. The time it took to complete the response was substantially increased in cells cultured in high-K^+ buffer but not when ChTX was used. The field potentials are expressed as mV ± S.E.M., and the temporal measurements are expressed as second ± S.E.M. (**P*<0.05, ***P*<0.01, ****P*<0.001, effects in normal *vs* High K^+ buffer; $^{\dagger}P$<0.05, $^{\dagger\dagger}P$<0.01, $^{\dagger\dagger\dagger}P$<0.001, effects in normal *vs* ChTX-containing buffer).

slight at concentrations ≤0.3 μg/ml, it nonetheless contributed to the overall field potential. For the sake of appropriateness, the term hyperpolarizing field potential (HFP) serves to denote the combined efflux of K^+ and the influx of Cl^- that attributed to the net recorded signal found in this study.

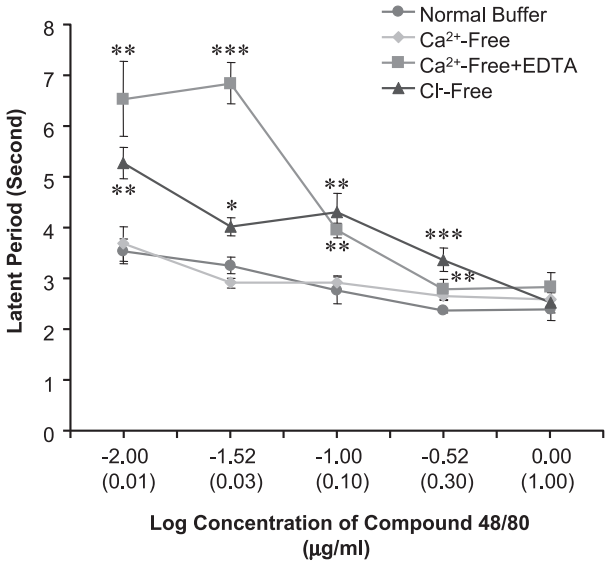

Fig. 4. Latent periods of compound 48/80 on peritoneal mast cells cultured in normal (n = 5 – 8), Ca^{2+}-free (n = 6 – 10), Ca^{2+}-free with EDTA (n = 4 – 7), and Cl^--free (n = 4) buffers. The latent periods were not different between cells that were cultured in normal and Ca^{2+}-free buffers. The inclusion of EDTA in Ca^{2+}-free buffer caused significant increases in latent periods at concentrations of up to 0.3 μg/ml. Chloride-free buffer also delayed the onset of the effect of compound 48/80 significantly. The results are expressed as the time (seconds ± S.E.M.) it took the compound 48/80 to first elicit an observable response from the moment of its administration. Differences in the latent periods in the presence of compound 48/80 (log concentrations and actual concentrations, μg/ml, in parentheses) with cells cultured in Ca^{2+}-free, Ca^{2+}-free with EDTA, and Cl^--free buffers were compared with those that were cultured in normal buffer (**P*<0.05, ***P*<0.01, ****P*<0.001).

Effects of compound 48/80 on peritoneal mast cells cultured in normal, Ca^{2+}-free, Ca^{2+}-free with EDTA, and Cl^--free buffers

Latent periods: The latent periods, the time it took compound 48/80 to elicit an observable effect, were similar in peritoneal mast cells in either normal or Ca^{2+}-free buffer between 0.01 – 1 μg/ml (normal: 2.37 ± 0.07 to 3.54 ± 0.24 s and Ca^{2+}-free: 2.59 ± 0.13 to 3.69 ± 0.34 s), but they were significantly increased at concentrations up to 0.3 μg/ml and were nearly doubled at 0.01 and 0.03 μg/ml when cells were in Ca^{2+}-free buffer containing EDTA (Fig. 4). Chloride-free buffer also significantly lengthened the latent period of compound 48/80 on peritoneal mast cells up to 0.3 μg/ml (Fig. 4). These differences in latency disappeared at the highest concentration of compound 48/80 (1.0 μM) regardless of the type of buffer used.

Spatial measurements: The HFPs of peritoneal mast cells were concentration-dependently increased in the presence of compound 48/80 (0.001 to 1 μg/ml). The increases in HFPs were significantly reduced at 0.3 and

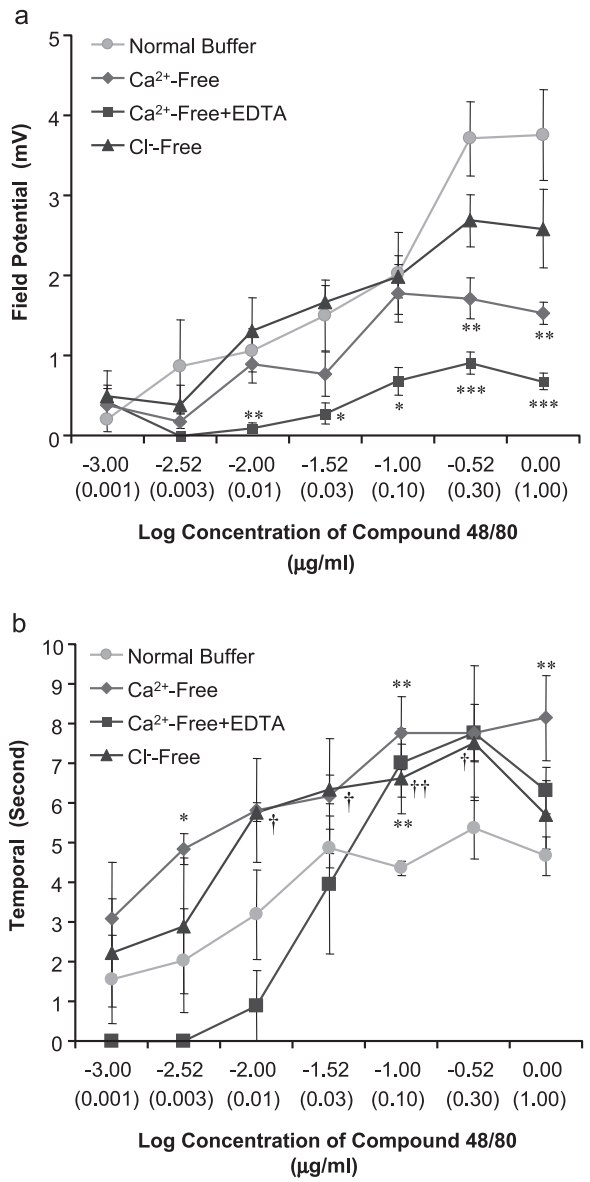

Fig. 5. Effects of compound 48/80 (the log concentrations and actual concentrations, μg/ml, in parentheses, n = 5 – 8) on HFPs (a) and temporal measurements (b) on peritoneal mast cells cultured in normal, Ca^{2+}-free, Ca^{2+}-free with EDTA, and Cl^--free buffers. Significant reductions in Ca^{2+} field potentials were found in cells cultured in Ca^{2+}-free buffer (n = 6 – 10) at high concentrations of compound 48/80 and even more so in Ca^{2+}-free buffer containing EDTA (n = 4 – 7). The time it took the response of compound 48/80 to complete was not hugely different in cells cultured in Ca^{2+}-free buffers with or without EDTA except at higher concentrations. The field potentials are expressed as mV ± S.E.M., and the temporal measurements are expressed as seconds ± S.E.M. (*P<0.05, **P<0.01, ***P<0.001). Although the HFPs of cells cultured in Cl^--free buffer in the presence of compound 48/80 were not significantly different, the time it took to complete its effect lengthened significantly ($^{\dagger}P$<0.05, $^{\dagger\dagger}P$<0.01, n = 4 – 5).

1 μg/ml when the peritoneal mast cells were exposed to Ca^{2+}-free buffer, and they were even more suppressed when extracellular free Ca^{2+} was further removed by the inclusion of EDTA in the Ca^{2+}-free buffer (Fig. 5a). The absence of extracellular Cl^- did not have any effect on HFPs upon the administration of compound 48/80 (normal: 0.21 ± 0.15 to 3.76 ± 0.56 mV and Cl^-: 0.50 ± 0.31 to 2.69 ± 0.33 mV). Although slightly lower potentials were observed at 0.3 and 1 μg/ml, these differences were not significant (Fig. 5a).

Temporal measurements

Compared with cells equilibrated in normal buffer, signals generally took longer to return to the baseline when cells were in Ca^{2+}-free and Ca^{2+}-free containing EDTA buffers (normal: 1.56 ± 1.11 to 5.37 ± 0.79 s, Ca^{2+}-free: 3.08 ± 1.42 to 8.15 ± 1.06 s, and Ca^{2+}-free containing EDTA: 3.94 ± 1.74 to 7.78 ± 1.70 s) (Fig. 5b). The relatively shorter recorded temporal measurements of cells in Ca^{2+}-free buffer containing EDTA at and below 0.01 μg/ml of compound 48/80 were because very low field potentials could be detected (0.10 ± 0.06 mV at 0.01 μg/ml), and recorded signals at these concentrations did not last very long (0 to 0.89 ± 0.89 s at 0.01 μg/ml). While compound 48/80 did not affect the HFPs of cells that were cultured in Cl^--free buffer, significantly longer temporal measurements were found between 0.01 – 0.3 μg/ml (Fig. 5b).

The changes in latent periods, field potentials, and temporal measurements of cells that were in these four buffers following the exposure to compound 48/80 are illustrated in Fig. 6.

Although partial removals of extracellular Ca^{2+} and Cl^- from normal buffer increased the time it took to complete the action of compound 48/80, these conditions alone did not seem to have a profound effect on HFPs. Further depletion of Ca^{2+} with the inclusion of EDTA significantly reduced the spatial and increased the temporal measurements, and this was also reflected by the EC_{50} values (the effective concentration that elicited 50% maximal spatial response in the presence of compound 48/80 on a given MEA). The EC_{50} of compound 48/80 was doubled in cells that were cultured in Ca^{2+}-free buffer containing EDTA, increasing from 0.027 ± 0.004 μg/ml in normal buffer to 0.072 ± 0.030 μg/ml in Ca^{2+}-free buffer containing EDTA (P<0.05, n = 4). No differences in the EC_{50}s of compound 48/80 were observed in cells that were cultured in Ca^{2+}-free and Cl^--free buffers (Fig. 7).

Discussion

The MEA system has been used mainly in the study of excitable cells such as the heart, brain, and the gastrointestinal tract. This is, however, the first time that the MEA has been used to measure extracellular field

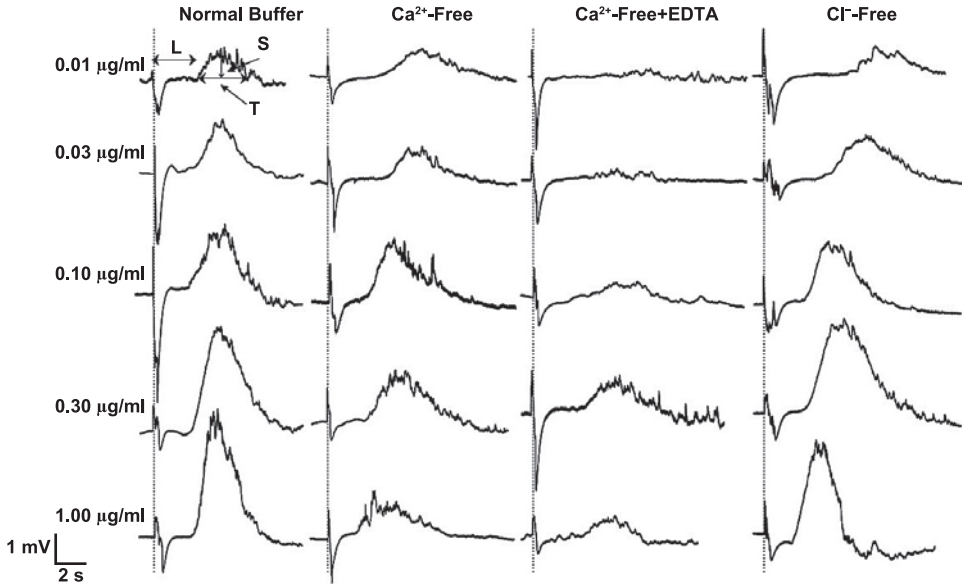

Fig. 6. Field potential recordings of peritoneal mast cells cultured in normal, Ca^{2+}-free, Ca^{2+}-free with EDTA, and Cl^--free buffers exposed to compound 48/80 (0.01 – 1.00 μg/ml). The latent period (L), spatial (S), and temporal (T) measurements are indicated. The large artefacts along the dotted lines indicate the moment when compound 48/80 was added. The HFP signal shapes revealed that the cell responses to compound 48/80 were progressively decreased as the amount of free Ca^{2+} present in the buffer decreased (signal strengths: normal buffer > Ca^{2+}-free buffer > Ca^{2+}-free plus EDTA buffer). At 0.01 μg/ml, compound 48/80 was only able to elicit a negligible hyperpolarization signal, and this signal virtually disappeared at 0.001 and 0.003 μg/ml (results not shown). Responses to the presence of this compound in cells that were cultured in Cl^--free buffer were affected substantially.

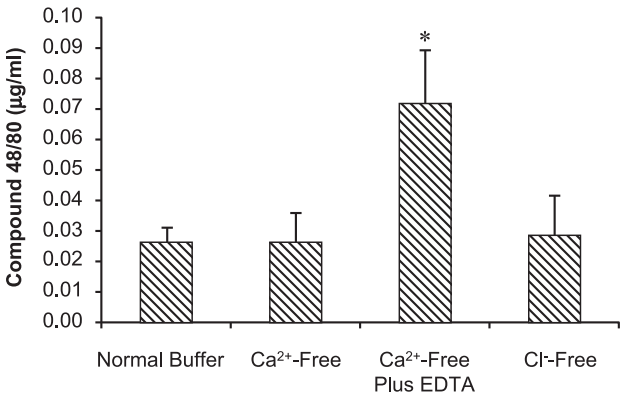

Fig. 7. The effective concentrations (EC_{50}s) of compound 48/80 on peritoneal mast cells cultured in normal, Ca^{2+}-free, Ca^{2+}-free with EDTA, and Cl^--free buffers. The results show that only cells that were cultured in EDTA-containing Ca^{2+}-free buffer were being affected by compound 48/80 significantly with a 3-fold increase in the average EC_{50} value (*P<0.05, n = 4).

potentials of rat peritoneal mast cells. Most currently available MEA systems, including our own, are only measuring field potentials of cardiac or neuronal cell monolayers in the order of several 100's of μV to 1 or 2 mV at best. It was therefore surprising to find that upon stimulation by compound 48/80, the recorded peritoneal mast cell field potentials were in the range of 4 to 6 mV and even up to more than 8 mV had been recorded in some occasions. This shows the present system is suitable for electrophysiological studies of non-excitable cells such as mast cells. Moreover, the fact that it could measure electrophysiological changes without doing so invasively means that the cellular homeostasis is preserved.

The data reveal that the recorded positive signal shape was comprised of at least two components: i) K^+ efflux, which probably includes the activation of inwardly rectifying K^+ and K_{Ca} channels, and ii) an additional hyperpolarizing potential that is unrelated to K^+ channel activation. The K^+ efflux component was partially inhibited by high K^+ or ChTX, and the remaining hyperpolarizing component was virtually completely eliminated when extracellular Cl^- was also removed from the ChTX-containing buffer. The present MEA system is probably the first to be able to monitor the combined changes of K^+ and Cl^- potentials of mast cells. It has been previously shown that our system offers a large bandwidth, especially in the low frequency band (36). This might be the reason why we were able to detect these signals from mast cells in the present study. For instance, oscillations between Ca^{2+} and K^+ might result in a positive shift from the baseline, if the K^+ currents are faster than the calcium currents. Because of the high pass filter effect of our system, such a situation would result in a positive baseline shift as long as the oscilla-

tions are present. Another possible signal source could be the activation of the sodium-potassium ATPase, which is known to mediate histamine release and to play a role in mast cell recovery (43). It has been described that the activation of the sodium-potassium ATPase is generating a net outward current I_P (44). The time course and amplitude of such currents, however, are not really matching our present observations with mast cells here. Alternative measurements are currently underway to clarify the point of signal generation from non-excitable cells. In this study, because of the combined hyperpolarizing components of the recorded signal, the term HFP was used to denote this net change in field potential.

It is known that the activation of peritoneal mast cells requires Ca^{2+} influx, and compound 48/80 induces the release of Ca^{2+} from intracellular stores (16). Since K^+ channel activation, namely the K_{Ca} current, has been proposed to affect mast cell activation and thus histamine release (12, 22, 45) and that K^+ current correlates with the activation of CRAC channels (11), it presented an ideal setting to demonstrate the detection of this K^+ signal upon stimulation by compound 48/80 under different physiological conditions using the MEA.

The present findings are in good agreement with the general understanding of the degranulation process of peritoneal mast cells. A reduction of extracellular Ca^{2+} limits the extent of Ca^{2+} entry and thus should reduce the HFP of which a large percentage was probably due to the activation of K_{Ca} based on the ChTX results. Results in the present study, however, show that the reduction of this potential upon the administration of compound 48/80 on peritoneal mast cells cultured in Ca^{2+}-free buffer was only limited, and it was not really significant until EDTA, a bivalent cation chelator, was also included in the buffer. This suggests that the peritoneal mast cells do not require a large amount of Ca^{2+} entry in order to trigger exocytosis in the presence of a secretagogue such as compound 48/80. This is interesting, as has been demonstrated in other studies, in that the activation of G-proteins in rat peritoneal mast cells by stable GTP-analogues leads to exocytosis even when $[Ca^{2+}]_i$ is very low (46, 47). The results here clearly support the understanding that secretion by compound 48/80 is not totally dependent on external Ca^{2+} (16, 17, 48).

Similarly, the removal of extracellular Cl^- should, in theory, depolarize the mast cell, thereby preventing Ca^{2+} influx as it is known that the Ca^{2+} current depends on cell membrane potential (V_m) (6, 49). In normal circumstances, the activation of outwardly rectifying Cl^- channels causes an influx of extracellular Cl^- and thus gives rise to a stable negative membrane potential in rat peritoneal mast cells; and it is this negative V_m that facilitates the influx of Ca^{2+} through membrane store-operated Ca^{2+} channels and subsequently exocytosis (6, 49). The use of Cl^--free buffer in the present study should have increased the V_m to a relatively positive value, thus reducing the driving force for Ca^{2+}, and the subsequent Ca^{2+} entry would have been significantly reduced. It was, however, not the case. The positive V_m due to the removal of extracellular Cl^- was probably only enough to lengthen the latent periods and to prolong the temporal measurements without affecting the HFPs significantly (Fig. 5: a and b). In addition, slight HFP reductions occurred only at high concentrations of compound 48/80 in the Cl^--free buffer experiment, suggesting that the hyperpolarizing component due to Cl^- influx is relatively less than K^+ efflux and that this influx of Cl^- only becomes more prominent at high concentrations, thus reinforcing the results obtained in experiments involving the use of ChTX and Cl^--free buffer (Fig. 2). The results again corroborate those obtained in Ca^{2+}-free conditions in that the absolute presence of extracellular free Ca^{2+} is not a necessity. It is true that extracellular Ca^{2+} is required in the normal process of K_{Ca}-channel activation and thus mast cell degranulation, but it has been shown that entry of sufficient Ca^{2+} can still occur even at a positive membrane potential (50). Indeed, although it is known that outwardly rectifying Cl^- channels can be activated by compound 48/80, they have been shown not to be essential for it to cause exocytosis in mast cells (51) and that compound 48/80-induced exocytosis is largely independent of extracellular Ca^{2+} influx (1). Taken together, the results from Ca^{2+}-free and Cl^--free conditions further substantiate the view that activation of G-proteins in the presence of compound 48/80 may not require the absolute presence of extracellular Ca^{2+} but, rather, involves the release of G-protein $\beta\gamma$-subunits (52, 53). The lengthening of the latent periods and temporal measurements was probably due to the reduction of the electrochemical gradient of Ca^{2+} as a direct result of an elevated V_m, thus affecting the onset and duration of the HFP. The effect on the Ca^{2+} electrochemical gradient alone, however, was not sufficient to prevent the effect of compound 48/80. The EC_{50} values also confirmed that the potency of compound 48/80 was not affected except when the peritoneal mast cells were cultured in EDTA-containing, Ca^{2+}-free buffer.

Based on the results obtained in this study and the available literature, it is clear that the sequence of events with regard to K^+ and Ca^{2+} is important to the understanding of mast cell activation. It seems very possible that Ca^{2+} entry, while not essential to stimulus-induced activation, is the first to occur via a non-receptor

mediated mechanism involving G-proteins. This entry of Ca^{2+} activates K_{Ca} channels, leading to an increase in outward K^+ current. This opening of K_{Ca} channel results in a negative shift in membrane potential, which in turn enhances Ca^{2+} influx by increasing the electrochemical driving force for Ca^{2+} entry and possibly even the CRAC current. In fact, a mast cell electrical 'excitation' cycle hypothesis based on human mast cells has been put forward by Bradding and Conley (14), suggesting something very similar in this regard. There is no doubt that the activation of mast cells requires co-ordinated activations of different ion channels and strict regulations of membrane potentials; thus, certain allergic responses may be controlled simply by modifying or controlling these critical processes.

In this study, the observed effects of peritoneal mast cells using the MEA are comparable and complementary to the existing knowledge obtained using other conventional means, including the patch-clamp technique. The application of patch-clamp in the study of mast cell electrophysiology is based on the assumption that the estimation of secretory activity of a single cell can be quantified by the cell membrane capacitance (24). Since one of the principles of the MEA is based on capacitive coupling between the cell and the electrode and this is then subsequently interpreted as field potentials based on the point contact model (42), the changes in field potentials can thus be correlated with the activation of these immunological cells. This study presented a novel way of interpreting electrophysiological activities of peritoneal mast cells other than using the patch-clamp which is very labor-intensive and difficult to perform on cells as small as the mast cell. The MEA system may even be applicable for studies on basophils, neutrophils, or other immunological cell types as they may also produce subtle electrophysiological changes that can be detected by the present system.

There are many benefits of using the MEA over the conventional patch-clamp technique. The MEA system is relatively cheap to maintain, and the chips are durable. The experiments are quick and easy to perform. The cells are not impaled; thus, the responses are stable and the data are highly reproducible. Furthermore, 64 cells can potentially be recorded simultaneously and this provides a better averaging of cellular responses. Indeed, we were able to record from 64 channels of a single chip in many occasions. In cases where the treatment given is reversible, these cell-integrated chips can be reused for other experiments as long as the cells are not infected or damaged and cell sensitization to a given drug has not occurred.

Since the MEA system can help to elucidate the response of mast cells to different drugs quickly, it can be used as a high-throughput means of characterizing anti-allergic drugs in the future. There is also the added benefit of reducing the number of animals used. In this study, more than 500 μl of 3×10^6 cells/ml of peritoneal mast cells could be obtained from a single rat. Since only about 10^5 cells were needed per chip (approximately 30 μl suspension per chip), we could potentially have more than 15 cell-seeded MEA chips from a single animal. In fact, since the recording area is very small, more cell-seeded chips can be obtained from each culture as only really just over 20 μl of this cell suspension is needed to cover the entire recording area of each MEA.

This study provided data that support some well-established knowledge of peritoneal mast cell activation, and it clearly demonstrated that the use of MEA in the study of non-excitable cells is possible. This system, however, is not without its drawbacks. Unlike the patch-clamp technique, this system cannot determine the types of receptors or channels that are present on the cell membrane unless some really specific agonists and antagonists are readily available. Nonetheless, it does provide a high-throughput means of studying immunological cells efficiently, which is not possible with standard electrophysiological techniques. When a rapid evaluation of certain cell types with regards to an immunological reaction is required, such as primary human mast cells, this system could be very useful. As previous analysis programs were designed for measuring cardiac and neuronal cells, which have very different signal shapes and responses, further work will now be focussed on the development of specific software that enables better, faster, and more comprehensive analysis of the many electrophysiological components that can be obtained following mast cell stimulations.

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