Modulatory action of potassium channel openers on field potential and histamine release from rat peritoneal mast cells

Chi-Kong Yeung, Jessica Ka-Yan Law, Sze-Wing Sam, Sven Ingebrandt, Hang-Yung Alaster Lau, John Anthony Rudd, and Mansun Chan

Abstract: To determine whether changes in membrane potential affect the extent of mast cell degranulation, compound 48/80 was added to rat peritoneal mast cell suspensions in the absence or presence of potassium channel openers (KCOs). Changes were compared between the field potential (FP) and the amount of histamine released. The results demonstrated that (*i*) the onset and duration of FP, which reflects the hyperpolarizing nature of the response, increased as the concentration of compound 48/80 increased; (*ii*) both FP and the amount of histamine released increased as the concentration of compound 48/80 increased; (*iii*) although both KCOs (SDZ PCO400, a benzopyran derivative, and P1060, a cyanoguanidine derivative) potentiated compound 48/80-induced increases in FP and histamine release, without compound 48/80, they had no effect on either parameter; (*iv*) both glibenclamide and charybdotoxin significantly attenuated the compound 48/80-induced increase in FP; and (*v*) glibenclamide was able to attenuate the KCO-induced potentiation of FP. The results show that drugs presumably causing hyperpolarization can affect histamine release from rat peritoneal mast cells. The effect of KCOs on compound 48/80-induced response appears to be potentiation in nature rather than synergism. It is possible that KCO hyperpolarizes the cell membrane, enhances Ca^{2+} influx, and thus increases histamine release. As such, selective blockers of K^+ channels may be useful for the treatment of immunological disorders.

Key words: potassium channels, histamine release, field potential, microelectrode array, rat peritoneal mast cells.

Résumé : On a comparé les modifications du potentiel de champ (PC) et de la quantité d'histamine libérée induites par l'ajout du composé 48/80, en absence et en présence d'ouvreurs des canaux potassiques (OCK), dans le but de déterminer si des modifications de potentiels membranaires peuvent influer sur la dégranulation des mastocytes péritonéaux chez le rat. Les résultats ont démontré que (*i*) le début et la durée du PC, qui reflètent la nature hyperpolarisante de la réponse, ont augmenté avec l'augmentation de la concentration du composé 48/80; (*ii*) les taux de PC et d'histamine libérée ont augmenté avec l'augmentation de la concentration du composé 48/80; (*iii*) les OCK, SDZ PCO400 et P1060 , ont potentialisé les augmentations de PC et de la libération d'histamine induites par le composé 48/80, mais ils n'ont eu aucun effet sur ces paramètres lorsqu'ils ont été utilisés seuls; (*iv*) le glibenclamide et la charybdotoxine ont atténué de manière significative l'augmentation du taux de PC induite par le composé 48/80; et (*v*) le glibenclamide a pu atténuer la potentialisation du PC induite par les OCK. Les résultats montrent que les médicaments qui provoquent une hyperpolarisation peuvent influer sur la libération d'histamine des mastocytes péritonéaux de rats. L'effet des OCK sur la réponse induite par le composé 48/80 semble davantage potentialisateur que synergique. Il est possible que l'OCK hyperpolarise la cellule, stimule l'influx de Ca^{2+} et ainsi augmente la libération d'histamine. Les bloqueurs sélectifs des canaux K^+ pourraient donc être utiles dans le traitement des désordres immunologiques.

Mots-clés : canaux potassique, libération d'histamine, potentiel de champ, réseau de microélectrodes, mastocytes péritonéaux de rats.

[Traduit par la Rédaction]

Introduction

Mast cells are involved in a number of pathophysiological allergic reactions such as type 1 allergic responses, which are typified by the exocytotic release of histamine and other mediators after immunological or nonimmunological challenges (Metcalfe et al. 1997). It has long been known that mast cell activation can be influenced by the physiological

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C.K. Yeung,[1] S.W. Sam, H.Y.A. Lau, and J.A. Rudd. School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong.
J.K.Y. Law and M. Chan. Department of Electronic and Computer Engineering, The Hong Kong University of Science and Technology, Hong Kong.
S. Ingebrandt. Department of Informatics and Microsystem Technology, University of Applied Sciences Kaiserslautern, D-66482 Zweibrücken, Germany.

[1]Corresponding author (e-mail: yeungck@rocketmail.com).

states of different ion channels, such as calcium (Ca^{2+}), potassium (K^+), and chloride (Cl^-) (Bradding 2005; Bradding and Conley 2002). Although the movement of Ca^{2+}, especially as a result of the activation of calcium release-activated calcium (CRAC) channels, plays an important role in mast cell activation (Bradding 2005; Hoth et al. 1993; Hoth and Penner 1993), the movement of K^+ can be just as important because it can affect the extent of Ca^{2+} entry and thus the release of downstream mediators (Bradding 2005; Bradding and Conley 2002).

Available evidence has revealed that the activation of outward K^+ current correlates with the activation of CRAC current in both time and amplitude in mast cells (Hoth 1996) and that the Ca^{2+}-dependent K^+ (K_{Ca}) channel current enhances the release of mediators (Duffy et al. 2001). Furthermore, in contrast to excitable cells, mast cell membrane hyperpolarization is required to support Ca^{2+} entry (Matthews et al. 1989). Indeed, compound 48/80, a synthetic nonpeptide secretagogue (Metcalfe et al. 1997), has been shown to cause a fast hyperpolarization and an increase in intracellular Ca^{2+} levels (Cabado et al. 1999). It thus appears that K^+ and Ca^{2+} are intrinsically linked, and that K^+ channel activation may be even more critical than the entry of Ca^{2+} in the control of mediator release. Drugs that specifically enhance the opening probability of K^+ channels, such as potassium channel openers (KCOs), could therefore affect the process of mast cell degranulation. Since the effects of such a secretagogue on mast cells can also be quantified by the amount of histamine released (Lau et al. 2001), it may be useful to correlate the subsequent release of histamine with the changes in ionic movements, in particular that of K^+.

In this study, the microelectrode array (MEA) was used to measure the field potentials (FPs) of rat peritoneal mast cells triggered by compound 48/80 alone as well as in the presence of different concentrations of two KCOs: SDZ PCO400 (SDZ), a benzopyran derivative, or P1060, a cyanoguanidine derivative. These KCOs were chosen because they are known to have an effect on adenosine triphosphate K^+ (K_{ATP}) channels (Fozard and Manley 2001) and because the activation of these channels has been implicated in immunological disorders (Chandy et al. 2004), such as asthma (Mannhold and Leclerc 2005). The changes in FP as a result of the application of compound 48/80 in the absence or presence of KCO were compared with the changes in the amount of histamine released after the same drug treatment protocol with the aim of determining whether K^+ channel activation, and hence membrane hyperpolarization, affects the extent of peritoneal mast cell degranulation. Furthermore, glibenclamide (GBC), which inhibits K_{ATP} channels, and charybdotoxin (ChTX), which inhibits K_{Ca} channels, were used to validate the type(s) of K^+ channel that may be present.

Materials and methods

Animals

Rat peritoneal mast cell suspensions were obtained from male Sprague–Dawley (SD) rats (400–450 g). The rats were outbred within the Laboratory Animal Services Centre of the Chinese University of Hong Kong and were housed at approximately 25 °C in a 12 h light : 12 h dark cycle. The Animal Experimentation Ethics Committee of the Chinese University of Hong Kong approved the experiments and protocols that were used.

Preparation of peritoneal mast cells

SD rats were killed by decapitation, followed by exsanguination under running water. Standard buffer containing 25 mL of heparin (50 IU/mL) was injected intraperitoneally. The solution-filled peritoneal cavity was then gently lavaged for 2 min. The injected buffer (containing approximately 4%–5% mast cells at this point) was extracted and centrifuged at 180*g* at 4 °C for 5 min.

After discarding the supernatant, the cells were resuspended in standard buffer (pH 7.4) containing (in mmol/L) NaCl 137, glucose 5.6, Hepes 10, KCl 2.7, NaH_2PO_4 0.4, and $CaCl_2$ 1.0, supplemented with BSA (1 mg/mL). Rat peritoneal mast cells were isolated and purified using a Percoll density gradient. A 1 mL aliquot of the cell suspension was mixed with 4 mL of 90% Percoll, and 1 mL of BSA-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. Mast cells gathered as a single layer at the bottom of the tube, whereas other cells formed a rather compact layer on top of the gradient. The mast cell pellet was then collected and washed twice in standard buffer by centrifugation and finally resuspended at the desired cell density. The mast cell purity of >98% was confirmed with toluidine blue staining.

Microelectrode arrays

The MEAs were manufactured on glass wafers (Schott Borofloat 33, Mainz, Germany) using standard silicon technology. The planar 64-channel gold MEAs (8 × 8) with a diameter of 20 or 30 μm were used at a pitch of either 100 or 200 μm. To use each MEA several times, the chip surface was passivated by an oxide–nitride–oxide layer deposited by plasma-enhanced chemical vapour 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 (Ecken et al. 2003; Krause 2000; Krause et al. 2000).

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, USA) was used, and it was then coupled to a main amplifier (gain 33×) providing an overall gain of 1089×. The high-pass performance of the recording system, which provided a large bandwidth recording, was dependent only on the size, material, surface condition, and cleanliness of the microelectrodes (Wrobel et al. 2007). As a result of this ultimate high-impedance input, the bandwidth (3 dB cutoff) of the recording system was enhanced to 1.7–3.7 kHz for planar gold microelectrodes with a diameter of 30 μm. This performance enabled reliable recordings of distinct signal shapes of extracellularly recorded FPs originating from individual cells. 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, Japan). 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 (Ecken et al. 2003; Krause 2000; Krause et al. 2000).

Electrophysiological measurements

Effect of KCOs on FP responses elicited by compound 48/80

Mast cells were resuspended at a cell density of approximately 3×10^6 cells/mL in standard buffer. A 33 µL aliquot of the cell suspension (about 10^5 cells) was placed onto the recording area of an MEA (Fig. 1*a*). 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 buffer (KCO-free control) or buffer containing the required concentration of KCO (0.01, 0.1, or 1 µmol/L) for 10 min incubation. A bolus concentration of compound 48/80 (10 µL of a particular concentration) was delivered by a microsyringe to give a final MEA volume of 200 µL. The final bath concentrations of compound 48/80 chosen (0.01–1.0 µg/mL) were comparable to the concentrations used in other studies. The microsyringe 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 compound 48/80 was added and the full electrophysiological response had occurred (~30 s), another cell-containing MEA was used. Other controls were performed by adding (*i*) 10 µL of drug-free standard buffers or vehicles of the KCO (≤0.004% ethanol) to cell-containing MEAs or (*ii*) 10 µL of compound 48/80-containing standard buffers to cell-free MEAs to ensure that no electrophysiological signals were generated by either the physical injection or presence of a drug.

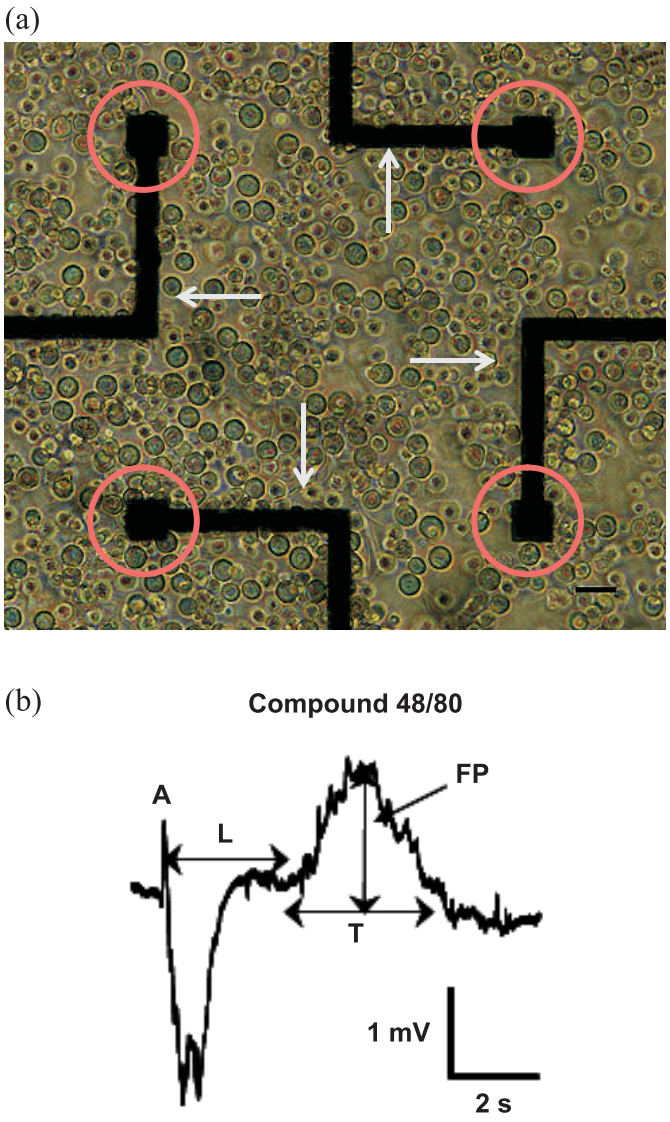
Effect of KCOs on the amount of histamine release elicited by compound 48/80

The release of histamine was elicited by adding compound 48/80 alone (0.01–1 µg/mL) or after 5 min incubation with KCO (at final concentrations of 0.001, 0.01, 0.1, or 1 µmol/L) or vehicle (≤0.004% ethanol *v*/*v*). Ten minutes after the addition of compound 48/80, 560 µL of cold standard buffer was added to stop the reaction. In the control group, the standard buffer was added at the addition step instead of compound 48/80.

Effect of KCOs on FP responses elicited by compound 48/80 in the presence of K^+ channel blockers

GBC (which inhibits K_{ATP} channels) and ChTX (which inhibits K_{Ca} channels) were used to determine the contribution of different types of K^+ channel activation relative to the total FP response upon the addition of compound 48/80 in the absence or presence of KCO. GBC (3 µmol/L) and ChTX (0.1 µmol/L) were equilibrated for 10 min before the addition of either SDZ or P1060 (0.01 µmol/L in each case). Compound 48/80 (0.001 µg/mL) was added 10 min thereafter. This lower concentration of compound 48/80 ensured that any potentiation of FP as a result of the presence of KCO was observed more readily.

Fig. 1. (*a*) Phase contrast image of the rat peritoneal mast cells seeded on a microelectrode array (MEA) was taken using an inverted microscope. Scale bar represents 30 µm. Four electrodes (circles) and their connecting lines (arrows) of an MEA are indicated. (*b*) A representative field potential recording of the peritoneal mast cells in the presence of compound 48/80 (0.01 µg/mL). The latent period (L), temporal period (T), and field potential (FP) measurements are indicated. The large artifact (A) is caused by the administration of compound 48/80.



Drugs and chemicals

SDZ ((–)-(3*S*,4*R*)-3,4-dihydro-3-hydroxy-2,2-dimethyl-4-(3-oxo-cyclopent-1-enyloxy)-2*H*-1-benzopyran-6-carbontrile) was a gift from Sandoz Pharmaceuticals. P1060 (*N*′-cyano-*N*-(3-pyridyl)-*N*″-(*t*-butyl) guanidine) was a gift from Leo Pharmaceuticals. Stock solutions of these KCOs (10 mmol/L) were made with 40% ethanol (*v*/*v*) and subsequently diluted with the standard buffer. Percoll was purchased from GE Healthcare Bioscience (Uppsala, Sweden) for gradient purification. Compound 48/80 ($C_{11}H_{15}NO$) and all other chemicals of analytic grade were purchased from Sigma-Aldrich, St. Louis, USA. The stock of GBC (20 mmol/L) was prepared by dissolving 30 mg in 3 mL of a solution

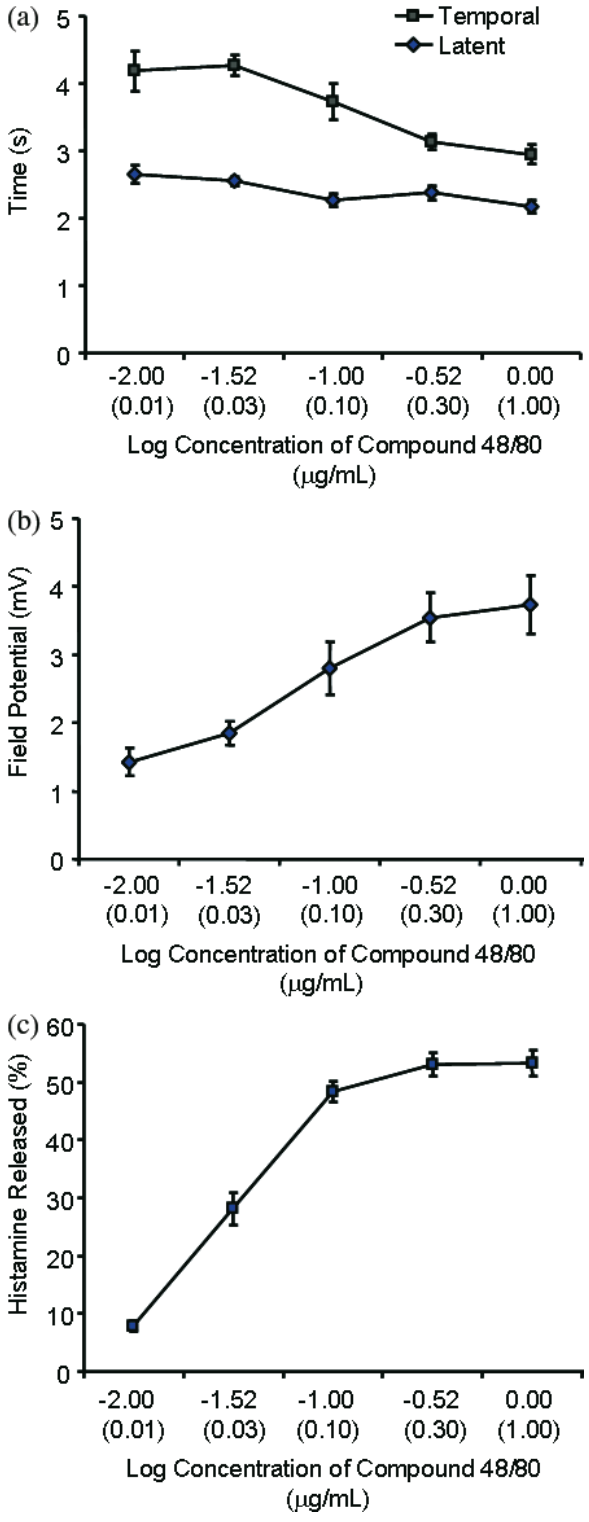


Fig. 2. Effect of compound 48/80 on latent (onset) and temporal (duration) time periods (*a*), field potential (*b*), and histamine release (*c*). Measurements are plotted against the logarithm of compound 48/80 concentration; actual concentrations (μg/mL) are shown in parentheses. Compound 48/80 caused a concentration-dependent reduction in the onset and duration of response, while the 'hyperpolarizing' field potential increased (n = 11–16). Compound 48/80 also increased the amount of histamine released in a concentration-dependent manner (n = 13–16).

containing 1.0 mL ethanol, 1.0 mL polyethylene glycol 400, 0.6 mL of 1 mol/L NaOH, and 0.4 mL distilled water. The stock of ChTX was made using distilled water. Subsequent dilutions of both blockers were made using the standard buffer.

Expression of results and statistics

MEA recording

The results are expressed in terms of (*i*) latent period (in seconds): the time from the moment of drug application to the onset of response, (*ii*) FP (in millivolts): the movement of different ions (cations and (or) anions) in and out of the mast cell, and (*iii*) temporal period (in seconds): the time for the FP recording to return to baseline. A representative trace of these parameters is shown in Fig. 1*b*. As the concentrations of compound 48/80 used were not linear, the logarithm of the concentrations is shown in the graphical presentation of the results. The above measurements were expressed as means ± standard error of the mean (SE), obtained from an average of >15 randomly selected channels from each MEA chip, and this average was considered as one experiment (n = 1).

Histamine release assay

The amount of histamine released was determined by fluorometric assay. The results were calculated as a percentage of the total histamine content released by the cells into the supernatant after exposure to compound 48/80:

$$\text{Histamine released } (\%) = \frac{S}{S+C} \times 100\%$$

where S is the amount of histamine released into the supernatant and C is the amount of histamine remaining in the cell pellet.

The percentage differences in the amount of histamine released after exposing the cells to a particular concentration of compound 48/80 in the absence or presence of KCOs were compared.

The MEA results were compared using 2-way ANOVA followed by Bonferroni t test or Student's unpaired t test, as appropriate. Values of $p < 0.05$ were considered to indicate a statistically significant difference. The histamine results were analyzed using Student's paired t test.

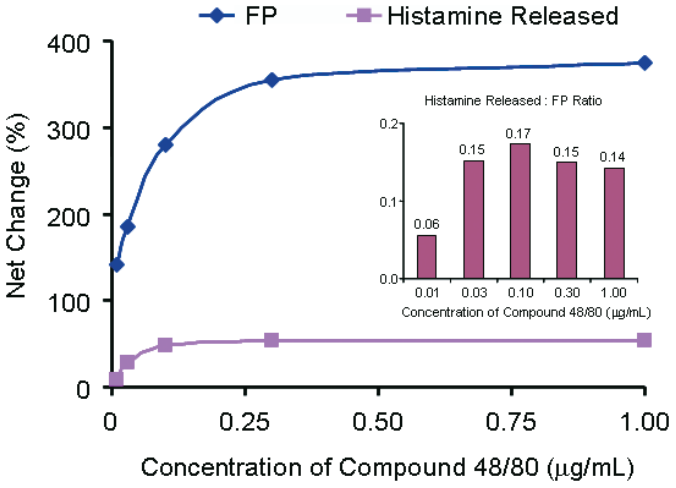
Results

The present study investigated the cell membrane hyperpolarization effect of compound 48/80 in the absence or presence of SDZ or P1060 and in relation to the amount of histamine released. To characterize the relative involvement of the types(s) of K^+ channels, ChTX and GBC were used to antagonize the hyperpolarizing effects of compound 48/80 alone and in the presence of either KCO.

Effect of compound 48/80 alone on peritoneal mast cells

Compound 48/80 alone produced no response when applied to the MEA directly, and no effect was observed when the mast cells were unstimulated. This lack of effect in the absence of drug was taken as zero. A concentration-

Fig. 3. As the 'hyperpolarizing' field potential (FP) increased, the amount of histamine released also increased over the entire concentration range of compound 48/80. However, the histamine-released-to-FP ratios (inset) revealed that there was less histamine released (>2.5-fold) with a given level of increase in FP at the lowest concentration of compound 48/80 (0.01 μg/mL) than at other concentrations. Ratios were calculated by dividing the percentage increase in the amount of histamine released (n = 13–16) by the percentage increase in FP (n = 11–14) at the corresponding concentrations of compound 48/80.

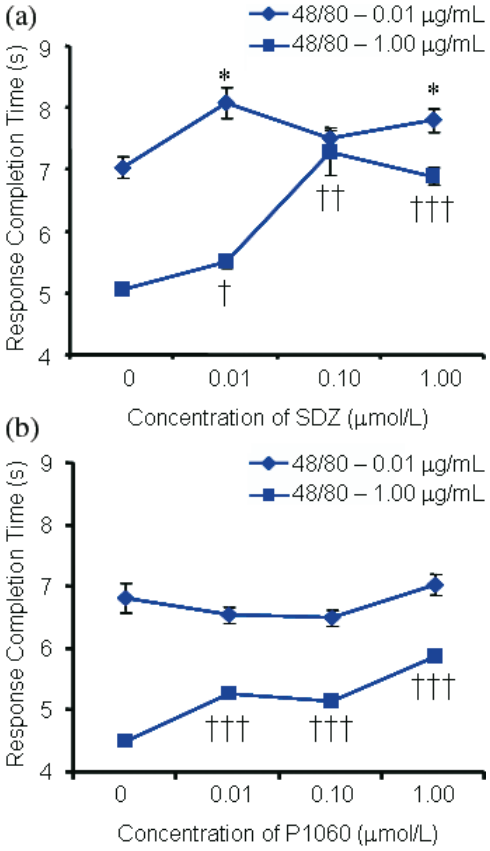


dependent reduction in the latent periods was observed upon the addition of compound 48/80 (0.01–1 μg/mL), reducing the time from 2.65 ± 0.13 s at 0.01 μg/mL (n = 13) to 2.17 ± 0.09 s at 1 μg/mL (n = 13), $p < 0.01$ (Fig. 2*a*). Similarly, the temporal measurements showed that the duration of response decreased from 4.19 ± 0.30 s at 0.01 μg/mL (n = 13) to 2.95 ± 0.15 s at 1 μg/mL (n = 13), $p < 0.001$ (Fig. 2*a*), while the FPs increased 2.6-fold from 1.42 ± 0.21 mV at 0.01 μg/mL (n = 12) to 3.74 ± 0.43 mV at 1 μg/mL (n = 11), $p < 0.001$ (Fig. 2*b*). The increase in the FP was reflected by a concentration-dependent elevation of the amount of histamine released that was observed at the same concentration range of compound 48/80, rising from 7.90% ± 0.87% at 0.01 μg/mL (n = 16) to 53.28% ± 2.20% at 1 μg/mL (n = 13), $p < 0.001$ (Fig. 2*c*). Because compound 48/80 alone elicited no resting FP, the recorded FP in its presence thus directly translated into the actual percentage increase from the resting state. This, in turn, aided a more direct comparison between the changes in FPs and the amounts of histamine released. Although the amount of histamine released increased as the FP increased, the histamine released-to-FP ratios revealed that the relative amount of histamine released was more than 2.5-fold lower at the lowest concentration of compound 48/80 (i.e., 0.01 μg/mL) than at other concentrations (Fig. 3, inset). The changes between the amounts of histamine released and FPs became more comparable at and above 0.03 μg/mL.

Effect of compound 48/80 on peritoneal mast cells in the presence of KCOs

Our previous study proposed that compound 48/80, especially at low concentrations, induces a net gain in FP that is related primarily to K^+ efflux or Cl^- influx (Yeung et al. 2008). As such, agents that open K^+ channels directly should, in principle, enhance this FP as well as the amount of histamine released. In the present study, therefore, SDZ and P1060 were used to determine whether their presence altered the overall response completion time (i.e., latent plus temporal time periods), the FP, and the amount of histamine released upon exposing the rat peritoneal mast cells to compound 48/80.

Fig. 4. Effect of potassium channel openers SDZ (*a*) or P1060 (*b*) on the response completion time (i.e., onset + duration of response) of compound 48/80 at 0.01 μg/mL and 1 μg/mL. Concentration-dependent lengthening of the response completion time of compound 48/80 was observed in the presence of both channel openers. *, significant at $p < 0.05$ compared with absence of SDZ at 0.01 μg/mL; †, significant at $p < 0.05$, ††, $p > 0.01$, and †††, $p < 0.001$ compared with absence of SDZ or P1060 at 1 μg/mL.



Response completion time

The time it took compound 48/80 to complete its effect (i.e., the response completion time) was obtained by combining the onset time (latent) with the duration of response (temporal) in the absence or presence of KCOs. Fig. 4 shows the effects of KCOs (0–1 μmol/L) on the lowest (0.01 μg/mL) and the highest (1 μg/mL) concentrations of compound 48/80. As before, response completion time of compound 48/80 alone was significantly longer at 0.01 μg/mL (6.89 ± 0.19 s, n = 13) than at 1 μg/mL (4.97 ± 0.06 s, n = 13), $p < 0.001$; however, the inclusion of either KCO lengthened this measurement in a concentration-related manner. Although SDZ (Fig. 4*a*) lengthened the response completion time elicited by both concentrations of compound 48/80, P1060 af-

Table 1. Change in field potential (mV ± SE) upon exposing peritoneal mast cells to compound 48/80 (0.01–1 μg/mL) in the presence of K^+ channel openers SDZ or P1060 (0.01–1 μmol/L).

	Compound 48/80, μg/mL				
	0.01	0.03	0.10	0.30	1.00
SDZ, μmol/L					
0.00	1.40±0.02	1.99±0.06	3.11±0.04	4.67±0.01	4.81±0.09
0.01	1.62±0.10 n.s.	2.04±0.03 n.s.	2.80±0.06 ↓	2.68±0.03 ↓↓↓	2.88±0.10 ↓↓↓
0.10	1.86±0.04 ↑↑↑	2.20±0.04 ↑	2.25±0.11 ↓↓	3.49±0.17 ↓↓	2.19±0.25 ↓↓↓
1.00	2.28±0.05 ↑↑↑	1.64±0.02 ↓↓	2.30±0.03 ↓↓↓	2.27±0.02 ↓↓↓	2.15±0.05 ↓↓↓
P1060, μmol/L					
0.00	2.08±0.03	2.26±0.11	4.81±0.20	4.44±0.15	4.59±0.29
0.01	2.74±0.06 ↑↑	2.56±0.13 n.s.	3.59±0.08 ↓↓	3.42±0.11 ↓↓	3.23±0.07 ↓↓
0.10	2.63±0.05 ↑↑	2.46±0.12 n.s.	3.50±0.06 ↓↓↓	3.51±0.14 ↓↓	3.15±0.07 ↓↓
1.00	2.25±0.23 n.s.	1.97±0.05 n.s.	2.46±0.02 ↓↓↓	2.47±0.05 ↓↓↓	2.24±0.04 ↓↓↓

Note: ↑, significant increase at $p < 0.05$, ↑↑, $p < 0.01$, and ↑↑↑, $p < 0.001$ in the 'hyperpolarizing' FPs elicited by compound 48/80 in the presence of KCO compared with results obtained in their absence ($n = 4$ or 5). Significant decreases in FPs in the presence of KCO are indicated with arrows pointing downward. n.s., not significant.

Table 2. Histamine released upon exposing peritoneal mast cells to compound 48/80 (0.01 μg/mL) in the presence of K^+ channel openers (KCOs) SDZ or P1060.

	Histamine released, %	
KCO, μmol/L	SDZ	P1060
0	7.90±0.87	7.90±0.87
0.001	12.97±2.17 ↑	17.10±4.04 ↑
0.010	13.40±2.11 ↑	15.68±4.10 ↑
0.100	11.14±1.95 ↑	16.73±4.45 ↑
1.000	10.20±1.74 n.s.	16.49±2.43 ↑

Note: ↑, significant at $p < 0.05$ compared with absence of KCO. The percentage of histamine released at 0.01 μg/mL of compound 48/80 alone ($n = 16$) was substantially increased in the presence of both KCOs (0.001–1 μmol/L, $n = 3$–7). But neither KCO increased the histamine released at concentrations of compound 48/80 exceeding 0.01 μg/mL (results not shown). n.s., not significant.

fected this aspect of cell response only at 1 μg/mL of compound 48/80 (Fig. 4*b*).

FP changes and histamine release

The addition of KCOs alone did not affect either the FP or the amount of histamine released. The changes in FP were largely dependent on the combination of a particular concentration of compound 48/80 and that of the KCO. Potentiation of FP in the presence of either KCO was mainly observed at low concentrations of compound 48/80 (up to 0.03 μg/mL). Interestingly, SDZ and P1060 actually reduced the recorded FPs when the concentration of compound 48/80 was at or above 0.1 μg/mL (Table 1).

The histamine data substantiated the FP data in that KCOs potentiated the release of histamine only when the concentration of compound 48/80 was 0.01 μg/mL (Table 2). There was neither potentiation nor inhibition of histamine released at other concentrations of compound 48/80 in the presence of either KCO (results not shown).

Effect of simultaneous presence of KCOs and K^+ channel blockers on compound 48/80-induced FP changes

ChTX (0.1 μmol/L) and GBC (3 μmol/L) were used to characterize whether the KCO-elicited increase in FPs was indeed due to the activation of K^+ channels. These concentrations were chosen because they provided a comparable level of inhibition. When given alone, each blocker significantly reduced the increase in FP triggered by the presence of compound 48/80 (0.01 μg/mL) (Fig. 5*a*). Combined treatment (GBC plus ChTX), however, did not yield a significantly larger inhibition than that obtained when either blocker was given alone ($p > 0.05$). In addition, although ChTX inhibited the effect of compound 48/80, only GBC ($p < 0.01$) was able to reduce the potentiation of FPs caused by the presence of either SDZ (Fig. 5*b*) or P1060 (Fig. 5*c*).

Discussion

Our previous study demonstrated the use of MEA in detecting a hyperpolarizing change in rat peritoneal mast cells upon the addition of compound 48/80 (Yeung et al. 2008). Our findings suggested that an increase in K^+ efflux is one of the most likely causes of membrane hyperpolarization, which subsequently leads to an eventual release of histamine after compound 48/80 challenge. Thus, the present study investigated 2 possible situations of K^+ channel activation in relation to rat peritoneal mast cell degranulation: (*i*) elevating the basal K^+ channel activity by the inclusion of KCOs alone triggers a release of histamine from rat peritoneal mast cells without the addition of compound 48/80, (*ii*) elevating the basal K^+ channel activity by the inclusion of KCOs enhances the amount of histamine released after the addition of compound 48/80.

There is a clear correlation between rat peritoneal mast cell electrophysiology and the amount of histamine released upon exposing the cells to compound 48/80 alone. The onset time and duration of response decreased as the concentration of compound 48/80 increased, whereas the FP and the amount of histamine released increased as the concentration of compound 48/80 increased.

The overall response completion time after the addition of compound 48/80 was prolonged in a concentration-related manner when the cells were preincubated with KCOs (Fig. 4). This suggests that an increase in K^+ channel opening probability and (or) duration in the presence of KCOs

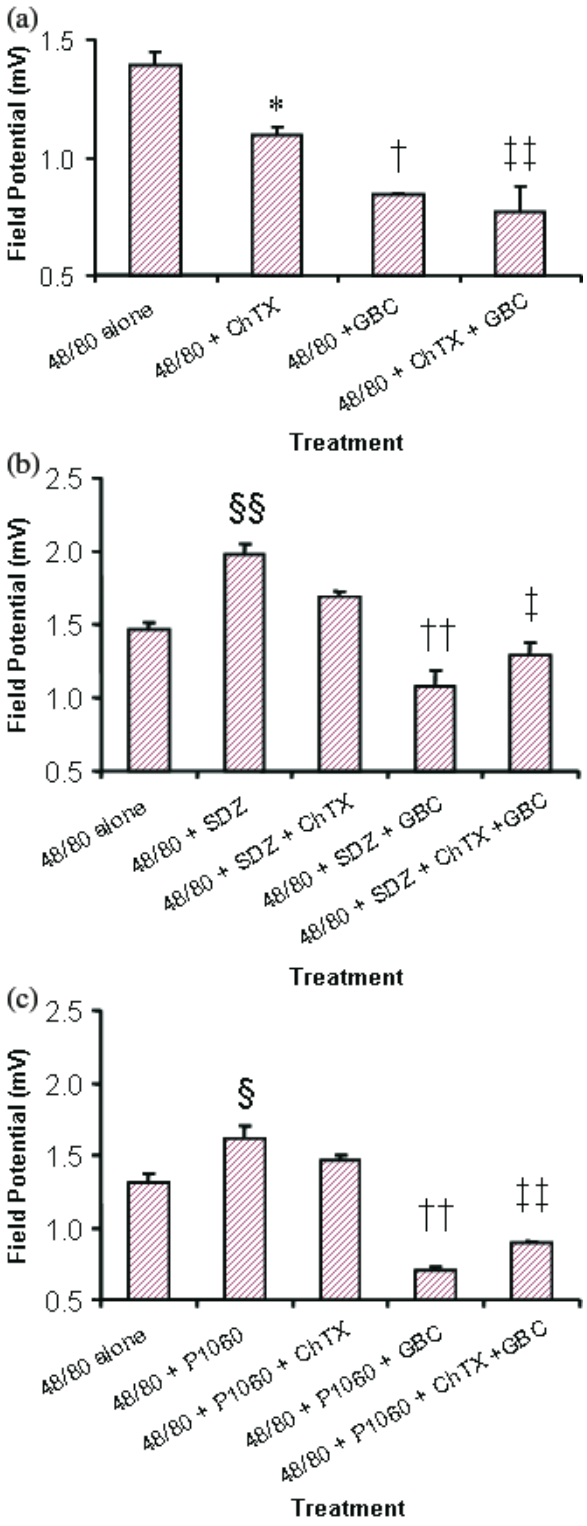


Fig. 5. Effect of charybdotoxin (ChTX) and glibenclamide (GBC) on the potentiation of compound 48/80-evoked FP response (*a*) as well as in the simultaneous presence of SDZ (*b*) or P1060 (*c*). Both SDZ and P1060 enhanced the compound 48/80-induced FP response (§, significant at $p < 0.05$, §§, $p > 0.001$). Although both ChTX (*, $p < 0.05$) and GBC (†, $p < 0.01$) were able to antagonize the compound 48/80-induced increase in FP response, ChTX alone did not reverse the KCO-induced potentiation of FP. Only GBC alone (††, $p < 0.001$) or in combination with ChTX (‡, $p < 0.01$, ‡‡, $p < 0.001$) significantly reduced the potentiation of FP by the presence of either KCO.

has a positive effect on the responses of cells in the presence of compound 48/80. Because both SDZ and P1060 were able to potentiate the FPs only at low concentrations of compound 48/80, however, means there must be a limit to how far this potentiation can go in the presence of KCOs. In other words, given that compound 48/80 alone already caused a certain level of increase in K^+ efflux and thus a certain level of FP change, a further increase in the concentrations of SDZ or P1060 did not induce a further rise in FP because the maximum hyperpolarizing potential, as a result of a net outward flow of positive ions, had already been reached (i.e., the net transmembrane K^+ flux was zero). It appears logical to assume that although KCOs may lead to a more prolonged K^+ channel opening duration over the whole concentration range of compound 48/80, they cannot enhance the efflux of K^+ beyond a certain electrochemical limit.

This 'FP limit' was also observed in the histamine release data. The control experiments showed that the increase in the amount of histamine released, when compared with a given magnitude of FP, was considerably lower at the lowest concentration than at other concentrations of compound 48/80, as indicated by the histamine released-to-FP ratios (Fig. 3). Since the lowest concentration of compound 48/80 did not yield a maximum or sufficiently large gain in the amount of histamine released on its own, the inclusion of KCOs probably hyperpolarized the cell membranes further and thus enhanced the amount of histamine released. As observed with the FP data, preincubation with KCOs (0.001–1 μmol/L) was able to elevate the amount of histamine released only at the lowest concentration of compound 48/80 (Table 2).

The presence of either KCO alone did not affect the FP or the amount of histamine released. This suggests that the activation of K^+ channels cannot in itself hyperpolarize the membrane of rat peritoneal mast cells to a point that actually promotes Ca^{2+} entry and thus degranulation. The fact that these KCOs alone did not trigger a rise in the background FP or the amount of histamine released suggests their effect on compound 48/80 was one of potentiation rather than synergism. It is possible that an initial incubation with a KCO 'resets' the resting membrane potential of cells to a more negative value (i.e., hyperpolarizing) thereby enhancing the influx of Ca^{2+} upon the addition of compound 48/80.

Another point to note is the amount of histamine released versus the extent of FP activation. It is known that cell membrane hyperpolarization, as a result of an efflux of K^+ ions, plays an important role in cell activation because it regulates membrane potential and thus Ca^{2+} influx and the subsequent cell depolarization and degranulation (Duffy et al. 2001; Lin et al. 1993; Mark Duffy et al. 2004). As discussed earlier, the amount of FP elevation appeared to be well correlated with the amount of histamine released barring the lowest concentration of compound 48/80 (Fig. 3). This suggests that there must be a critical point below which membrane hyperpolarization may not cause a 'sufficient'

change in Ca^{2+} influx despite an increase in K^+ efflux, and that degranulation will not occur unless this threshold level has been reached. Indeed, a study has shown that compound 48/80 could depolarize peritoneal mast cells without necessarily undergoing degranulation (Nakayama et al. 2002).

The present results appear to suggest that rat peritoneal mast cell membrane hyperpolarization in the presence of compound 48/80 is due, at least in part, to the activation of at least 2 major K^+ channels, namely K_{ATP} and K_{Ca} channels. But because GBC also inhibits Cl^- channels, such as swelling-activated, Ca^{2+}-activated, and cystic fibrosis transmembrane regulator (CFTR) (Sheppard and Robinson 1997; Yamazaki and Hume 1997), there is the possibility that the observed effects of compound 48/80 on mast cells are not solely due to K_{ATP} and K_{Ca} channel activation. The K^+ channel potentiation effect upon the addition of SDZ or P1060 was primarily the result of opening the K_{ATP} channels, since the effects of the KCOs were blocked by GBC, and ChTX alone only marginally decreased this potentiation in FPs (Fig. 5). The observed changes in FP may yet be found to involve movement of ions other than K^+ (such as Cl^-); nevertheless, this study shows that K^+ efflux-related membrane hyperpolarization could play an important role in mast cell activation. Because K^+ channel activation can potentiate the process of degranulation, the question is whether it is possible to block or inhibit K^+ channels such that the subsequent processes that lead to degranulation can be attenuated or even prevented from occurring altogether. It is known that K^+ channels, such as the voltage-gated $K_v1.3$ and K_{Ca} channels, regulate Ca^{2+} signalling via the control of membrane potential (Cahalan et al. 2001; Wulff et al. 2003). Since the activation of $K_v1.3$ channels (in response to membrane depolarization) and the opening of K_{Ca} channels (as a result of cytosolic Ca^{2+} elevation) depend on the state of immune cells, selective inhibitors of these channels may be useful for treating a number of immunological disorders (Chandy et al. 2004).

Further studies are currently underway to examine this possibility in the hope that treatments of allergic responses can be based upon controlling the release of mediators rather than simply antagonizing the presence of symptoms. Immunologically induced mediator release from mast cells by the ligation of high-affinity IgE receptors is known to be more dependent on the influx of extracellular Ca^{2+} and thus is more likely to be significantly regulated by K^+ channel modulators (Lau et al. 2001). At present, disodium cromoglycate is one of the mast cell stabilizers that are known to prevent the release of histamine (Shin et al. 2004). But if ionic movements, especially that of K^+, can be regulated, the severity of allergic responses involving histamine release may be more effectively controlled by using K^+ channel blockers as a 'new' form of mast cell stabilizer. In the meantime, it may be useful to precisely determine the overall contribution of K^+ channels involved in the regulation of FP change upon challenge by various secretagogues that are involved in the membrane hyperpolarization of peritoneal mast cells.

Conclusion

This study shows that the extent of rat peritoneal mast cell degranulation could be modulated, albeit within a strictly delimited concentration range, by the use of K^+ channel modulators. Our findings open up the possibility of controlling the release of histamine and (or) other mediators from mast cells by modifying K^+ channel status via selective blockade. This prophylactic approach may provide better clinical outcomes than simply treating the symptoms that are associated with allergic responses.

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