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Noise of Secretagogue-Induced Inward Currents Dependent on Extracellular Calcium in Rat Mast Cells

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Summary. We analyzed the noise of the inward currents induced by stimulation of rat peritoneal mast cells with compound 48/80 (48/80), a secretagogue, and examined the role of extracellular Ca^{2+} in generation of the large noise. In the presence of 2 mM Ca^{2+} in the external solution, the power density spectra of the 48/80-induced inward currents in most cells were fitted with the sum of two Lorentzian functions. The cut-off frequencies (f_c) at −50 mV for the low and high frequency components were 16.3 ± 7.3 (n = 10) and 180 ± 95 (n = 9) Hz. Involvement of a cation-selective channel in the large noise was identified in some cells, but the single channel current amplitude estimated from parameters of the noise varied among cells (0.20–2.47 pA at −50 mV), thereby indicating that the currents were mediated by more than two classes of channel. The low frequency component of the 48/80-induced currents was suppressed by lowering the extracellular Ca^{2+} concentration to 1 μM with the addition of EGTA, without appreciable changes in the high frequency component. When the extracellular Ca^{2+} was reduced to 1 μM by EGTA 1 min prior to stimulation, 48/80 induced little or no currents in most cells and small currents in some cells. The power density spectra of the small currents were fitted mainly by a single Lorentzian curve with an f_c of 150 ± 5.8 Hz (n = 3). Re-admission of 1.3 mM Ca^{2+} produced a low frequency part of current noise with an f_c of 18.8 (n = 2) Hz. When the extracellular Na^+ was totally replaced by N-methyl-D-glucamine or choline in the presence of 2–5 mM Ca^{2+}, the mean current amplitude was smaller than that in the Na^+-containing medium, but the power density spectra of the current noise were fitted by a sum of two Lorentzians with f_c of 13.7 ± 6.4 (n = 6) and 186 ± 77 (n = 6) Hz. These results suggest that low frequency fluctuation of currents depends on the extracellular Ca^{2+} and underlies the large noise of the 48/80-induced inward currents. The 48/80-induced Ca^{2+} influx seems to be essential to generate the low frequency fluctuations, and Na^+ influx through the cation-selective channel would augment the amplitude of the fluctuation.

Key Words receptor-operated Ca^{2+} influx · compound 48/80 · mast cell · current noise · stimulus-secretion coupling

Introduction

When antigens or nonspecific secretagogues come into contact with mast cells various chemical mediators are secreted, and during this stimulus-secretion coupling in mast cells several ion channels are activated via second messengers (Penner, Matthews & Neher, 1988; Kuno, Okada & Shibata 1989; Matthews, Neher & Penner, 1989*a*; Kuno et al., 1990). Patch-clamp studies revealed that stimulation with the secretagogue, compound 48/80 (48/80), induced inward currents characterized by a large noise (Kuno et al., 1989; Matthews et al., 1989*a*). As the significance and mechanisms of this large noise has remained to be defined, we analyzed the noise of the 48/80-induced inward currents and obtained evidence that the noise was composed of low and high frequency components and that the large noise was due to a low frequency (8–30 Hz) current fluctuation which depended on extracellular Ca^{2+}. We suggest that the influx of Ca^{2+} is essential to generate the low frequency component and that Na^+ influx through the cation-selective channel would augment the amplitude of the current fluctuation. A preliminary abstract of this work was published (Kuno & Kimura, 1991).

Materials and Methods

Cells

Male Wistar rats (~350 g) were killed by an overdose of ether, and Ringer solution was injected into the peritoneal cavity. The peritoneal cells including mast cells were collected by aspiration, washed twice with Ringer solution by centrifugation at 800–1000 rpm. The cells were resuspended in fresh Ringer solution and transferred into a small recording chamber. The standard Ringer solution contained (in mM): 150 NaCl, 2.8 KCl, 1 or 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES and 0.1% bovine serum albumin. The pH was adjusted to 7.3. All experiments were carried out at room temperature (18–24°C).

External and Internal Solutions

The standard Ringer (*see* above) was used as the external solution unless stated otherwise. In the Na^+-free solutions, Na^+ was replaced by 150 mM of N-methyl-D-glucamine or choline. The exter-

nal Ca^{2+} was reduced by adding EGTA (ethyleneglycol *bis*-N,N,N′,N′-tetraacetic acid) to the solutions: In the presence of EGTA, the Ca^{2+} concentration was estimated by a calculation program for the Ca-EGTA mixture (Oiki & Okada, 1987). In whole cell clamp recordings, the patch pipette contained either an isotonic CsCl (160 mM CsCl, 1 mM $CaCl_2$, 11 mM EGTA and 10 mM HEPES-KOH, pH 7.3) or a K-glutamate (150 mM K-glutamate, 7 mM $MgCl_2$, 1 mM EGTA and 10 mM HEPES-KOH, pH 7.3) solution. In some experiments, 10–20 μM of 4,4′-diisothiocyano-2,2′-stilbenedisulphonate (DIDS), a blocker of Cl^- channel (Matthews et al., 1989*b*), was added to the solutions. With the CsCl solution, the cell often swelled and the conductance was increased within the first few minutes after rupture of the patch membrane. The input conductance then stabilized. We discarded cells in which the input conductance continued to increase for 5–10 min before stimulation. Similar results were obtained with pipettes containing CsCl or K-glutamate. In most cells, degranulation was inhibited by dialyzing cells with pipette solution lacking GTP (Linadu & Fernandez, 1986), and degranulated cells were eliminated from the noise analysis to avoid involvement of changes in conductances due to fusion of secretory granules into the plasma membrane. The liquid junction potentials at the pipette tip, measured as reported by Fenwick et al. (Fenwick, Marty & Neher, 1982), were ~2 mV (positive in pipette) for the CsCl and ~ −12 mV (negative in pipette) for the K-glutamate solutions. The zero current potential before the giga seal formed was taken as zero mV and a further correction was not made during each experiment.

Recordings

Current signals were recorded in whole cell configurations using a conventional patch-clamp technique (Hamill et al., 1981). Pipette resistances ranged between 5 and 10 MΩ. The current signals were led to a patch-clamp amplifier (CEZ2100, Nihon Kohden, Japan) without series resistance compensation and were stored on a FM tape recorder (TEAC XR-30H, Japan; band pass, DC-10 kHz). Current signals, filtered at 1 kHz, were displayed on a chart recorder with a characteristic frequency of 1 kHz (Nihon Kohden, RM-6000, Japan).

Current Noise Analysis

The currents were low-pass filtered at 5 kHz by a filter with Bessel characteristics with 48 dB/octave slope attenuation (NF, P83, Japan), digitized at 10 kHz by an A-D converter (ANALOG PRO I, Canopus), and were stored on a personal computer (NEC, PC9801VM, Japan). Some data were high-pass filtered at 0.2 Hz before digitization. The one-sided power density spectrum was calculated using a fast Fourier transformation (FFT) (Anderson & Stevens, 1973; Colquhon, Dreyer & Sheridan, 1979) for a frame of 8192 points and 5–10 frames were averaged. The sections including currents evoked by voltage pulses were removed from the analysis. The resolution of frequency was 1.22 Hz (10 kHz sampling, 8192 points). The averaged background spectrum in the absence of agonists was subtracted, and the net spectrum was displayed in log-log coordinates and fitted with Lorentzian functions of the form

$$S(f) = S(0)/\{1 + (f/f_c)^2\}$$

where, $S(f)$ is the spectral density at frequency f, $S(0)$ is the zero frequency asymptote and f_c is the cut-off frequency at which the spectral density falls to half of $S(0)$. The spectrum was fitted with either one or the sum of two Lorentzian functions, to minimize $\Delta \equiv \Sigma\,(P(f) - S(f))^2$ by successive iteration of the least squares method, where $P(f)$ is a raw power density spectrum at frequency f. The low and high f_c of the two Lorentzians were designated as f_{CL} and f_{CH}, respectively. The current variances were estimated directly from the data or from the parameters of the Lorentzian fit, and there was good agreement between the two estimates of variance. The variances from direct measurements were calculated in the presence and absence of 48/80 and then subtracted. The total current variance (var) and the current variances due to the low and high frequency components (var_L and var_H) were calculated from parameters of the Lorentzian fit for net spectra as follows. $var = \pi \cdot \{S_L(0) \cdot f_{CL} + S_H(0) \cdot f_{CH}\}$; $var_L = \pi \cdot S_L(0) \cdot f_{CL}$; $var_H = \pi \cdot S_H(0) \cdot f_{CH}$; $S_L(0)$ and $S_H(0)$ are zero-frequency asymptotes of the Lorentzian fits for the two components. The single channel current amplitude (i) was estimated from the mean current amplitude (I) and variance (var) as: $i = var/I$, when the linearity of the plots of var *vs.* I was confirmed (Stevens, 1972; Anderson & Stevens, 1973; Cull-Candy, Howe & Ogden, 1988): In each cell, I and var were obtained from direct measurements of records of 1.63 sec at various times during the gradual development of currents following stimulation to get points over a wide range, and var was plotted against I (*see* Figs. 3 and 9*b*). The var/I was calculated from data used for noise analysis, such as currents during the quasi steady state at near maximum, only in cases where the correlation coefficient for the linearity was larger than 0.8. Histograms of current amplitude were made for records of 6–13 sec using an analysis program (SHIST; written by T. Eguchi).

Chemicals

Compounds 48/80 (Sigma), DIDS (Sigma), EGTA (Sigma), $CaCl_2$, and $CdCl_2$ were kept in condensed stock solution. A small aliquot (10–50 μl) of the stock solution was applied in the vicinity of the cell, to obtain the final concentration indicated. The pH of EGTA stock solution was previously adjusted to 7.3 with NaOH.

Results

Noise Analysis of 48/80-Induced Inward Currents

Stimulation of mast cells with 48/80 induced inward currents and an increase in conductance (Penner et al., 1988; Kuno et al., 1989; Matthews et al., 1989*a*). In most cells, the inward currents appeared within 10–30 sec after stimulation, reached a peak around 1–2 min, and then gradually declined. The whole cell inward currents activated by 48/80 at −50 mV in the presence of 2 mM Ca^{2+} were characterized by significant noise and were blocked by Cd (Fig. 1). The power density spectrum of the 48/80-induced currents during the quasi steady-state portion around the peak response was fitted by the sum of two Lorentzian curves (Fig. 2). The cut-off frequen-

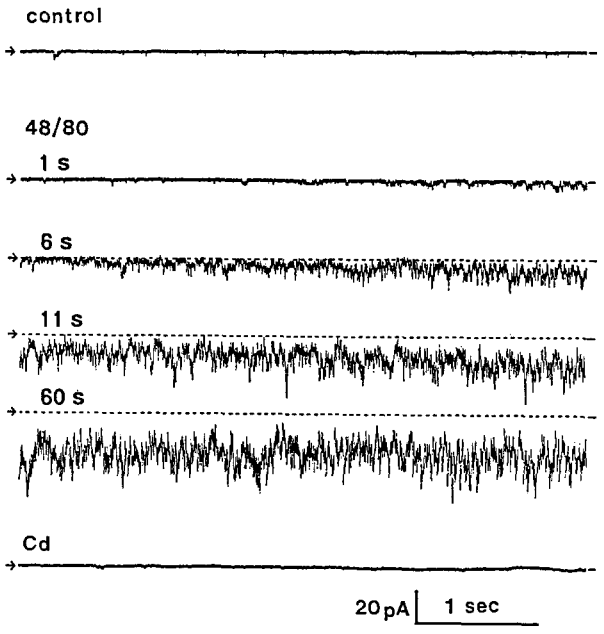

Fig. 1. 48/80-induced currents at −50 mV in the whole cell configuration. Traces are in control, various times after stimulation with 48/80 (3.3 μg/ml) and after addition of Cd (0.33 mM). The starting time of each record after addition of 48/80 is shown. Current signals were low-pass filtered at 1 kHz. Arrows indicate the level of zero current. Data were obtained from the No. 2 cell in the Table. The external medium contained 150 mM Na^+ and 2 mM Ca^{2+} and the internal medium contained CsCl.

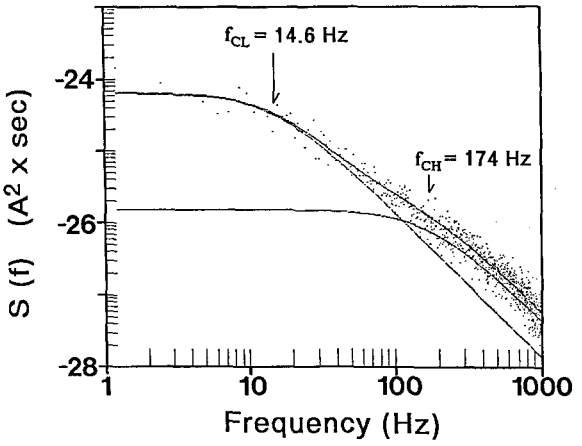

Fig. 2. The power density spectrum of 48/80-induced whole cell currents at −50 mV during the quasi steady state at near maximum response. Net spectrum was obtained by subtraction of the averages of spectra in the presence and absence of 48/80. Lines represent fits for two Lorentzian components, with f_c of 14.6 and 174 Hz, and the sum of the two curves. Data were obtained from the same cell in Fig. 1 (No. 2 cell in the Table). Total current variance estimated from the two Lorentzian components was 38.5 pA^2. The external medium contained 150 mM Na^+ and 2 mM Ca^{2+} and the internal medium contained CsCl.

cies (f_c) of the 48/80-induced whole cell inward currents for 12 cells in the presence of 1 or 2 mM Ca^{2+} are summarized in the Table. In 11 cells, the current noise was composed of two components, and the high frequency part was absent in cell No. 10. The low and high f_c (f_{CL} and f_{CH}, respectively) at −50 mV and in 2 mM Ca^{2+} were 16.3 ± 7.3 Hz (mean ± SD, n = 10) and 180 ± 95 Hz (n = 9). The f_{CL} and f_{CH} were not significantly different with the CsCl and K-glutamate internal solutions. The power density spectra of currents at −100 mV or in 1 mM Ca^{2+} was also fitted by two Lorentzians (Nos. 7 and 12). In later experiments, inward currents recorded at −50 mV were analyzed.

In the presence of 2 mM Ca^{2+}, the mean current amplitude of the 48/80-induced inward currents at −50 mV varied among cells used for the analysis, 36.1 ± 32.6 pA (n = 9). The relationship between the mean current amplitude and total current variance during the gradual development of currents following the addition of 48/80 in two cells is shown in Fig. 3. The total current variance divided by the mean current amplitude when the two parameters correlated closely ranged from 0.20 to 2.47 pA (1.31 ± 0.81, n = 6) at −50 mV and in 2 mM Ca^{2+} in six cells (Table), thereby suggesting that the 48/80-induced inward current was not composed of a single unitary current.

Effects of Extracellular Ca^{2+} on the 48/80-Induced Current Noise

Lowering the extracellular Ca^{2+} to about 1 μM by adding EGTA reduced the amplitude of the 48/80-induced inward currents, as previously reported (Kuno et al., 1989) (Fig. 4). Figure 4*b* shows the underlying slow fluctuations of the 48/80-induced currents in the presence of 2 mM Ca^{2+} after low-pass filtering of currents at 50 Hz (second traces in *b*); these findings are consistent with the low frequency component of the power density spectrum. Lowering Ca^{2+} from 2 mM to about 1 μM by EGTA reduced the mean amplitude and SD of the noise (Fig. 4*c*, bottom). The slow fluctuations of the 48/80-induced inward currents were reduced by addition of EGTA (Fig, 4*c*, middle) though the higher frequency noise remained (Fig. 4*c*, top). Superimposition of the power density spectra of the 48/80-induced currents immediately before and after the addition of EGTA (Fig. 5*a*) showed the selective reduction of the low frequency portion of the noise by EGTA. The power density spectrum of the noise that remained after the addition of EGTA was well fitted by a single Lorentzian with a f_c of 172 Hz (Fig. 5*a*). The difference spectrum between that of the 48/80-induced

Table. Parameters of power density spectrum of 48/80-induced inward currents

Cell	$[Ca^{2+}]_e$ (mM)	Pipette	H.p. (mV)	48/80-induced whole cell currents			
				f_{CL} (Hz)	f_{CH} (Hz)	var/I (pA)	var_L/var_H
1	2	Cs	−50	11.7	128	2.47	1.17
2	2	Cs	−50	14.6	174	1.23	3.69
3	2	Cs	−50	8.6	64	0.96	1.69
4	2	Cs	−50	27.0	256	0.98	1.93
5	2	Cs	−50	25.0	149	—	1.46
6	2	Cs	−50	22.4	269	—	3.62
7	2	Cs	−100	28.7	164	1.78	2.21
8	2	K-glut	−50	8.9	93	—	1.94
9	2	K-glut	−50	9.3	357	0.20	0.14
10	2	K-glut	−50	12.2	±	—	—
11	2	K-glut	−50	23.2	128	2.02	1.82
12	1	K-glut	−50	14.9	109	—	1.56

Analysis was conducted on inward currents during the quasi steady state at near maximum. f_{CL} and f_{CH} are cut-off frequencies of the low and high frequency components and variances of the total current and the low and high frequency components (var_L and var_H) were estimated from parameters of Lorentzian fits. "*var/I*" is the estimate of single channel current amplitude calculated from the total curret variance (var) divided by mean current amplitude (*I*) (*see* Materials and Methods) in cases where the linearity between variance and "*I*" was confirmed ($r > 0.8$). var_L/var_H is ratio of variances calculated for the two components. 48/80: 5 μg/ml. H.P.; holding potential. $[Ca^{2+}]_e$; the concentration of extracellular Ca^{2+}. The external medium contained 150 mM Na^+.

currents, before and that after addition of EGTA (48/80-EGTA) was fitted by a single Lorentzian curve with a f_c of 25.9 Hz (Fig. 5*b*). The average f_c of the Ca^{2+}-dependent noise in five cells, obtained from the difference power density spectra (48/80-EGTA), was 21.6 ± 6.8 Hz, with a reduction in mean current amplitude by 56 ± 13%.

Addition of EGTA prior to stimulation inhibited the 48/80-induced currents, but small inward currents were occasionally observed in some cells (Fig. 6*b*) (Kuno et al., 1989). Re-admission of Ca^{2+} increased the amplitudes of the mean current and the fluctuation (Fig. 6*c*), and these effects were suppressed by Cd (0.5 mM) (Fig. 6*d*). The power density spectrum of the 48/80-induced inward currents when EGTA was added 1 min before stimulation was fitted by a single Lorentzian curve (Fig. 7*a*). The average and SD of the f_c in three cells was 150 ± 5.8 Hz. Re-admission of Ca^{2+} (1.3 mM) at 5 min after stimulation with 48/80 induced current noise with low f_c, 18.8 Hz ($n = 2$) (Fig. 7*b*).

The ratio of the current variances due to the low and high frequency components, var_L and var_H calculated using parameters of the Lorentzian fit (*see* Materials and Methods), ranged from 0.14 to 3.69 in the presence of 2 mM Ca^{2+} (Table). The ratio was thus variable among cells but decreased when the extracellular Ca^{2+} was lowered. The average and SD of the ratio in the presence of 2 mM, 0.1 mM and 1 μM Ca^{2+} was 1.97 ± 1.06 ($n = 10$), 0.43 ± 0.27

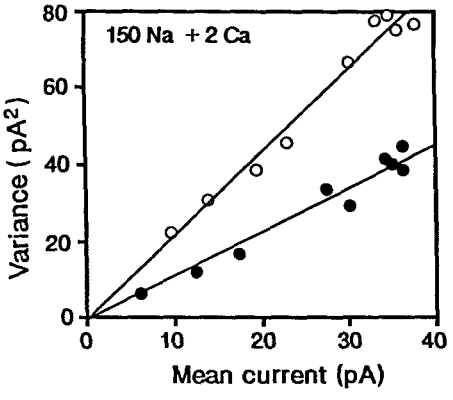

Fig. 3. Relationship between mean amplitude and total current variance of the 48/80-induced inward currents at −50 mV in two cells. Total current variance was calculated directly from data. Each point represents the values obtained from data during the development of the currents following stimulation in the No. 4 (filled circles) and No. 11 (open circles) cells in the Table. Lines indicate linear regressions of data, the slope of which was 1.14 pA (filled circles; $r = 0.96$) and 2.17 pA (open circles; $r = 0.97$), respectively. 48/80: 5 μg/ml. The external medium contained 150 mM Na^+ and 2 mM Ca^{2+} and the internal medium contained CsCl (filled circles) or K-glutamate (open circles).

($n = 4$) and 0.09 ± 0.10 ($n = 7$). These results suggest that the low frequency component of the 48/80-induced current noise underlies the large noise and depends on extracellular Ca^{2+}.

48/80 has been reported to increase openings of a cation-selective 30–50 pS channel without appre-

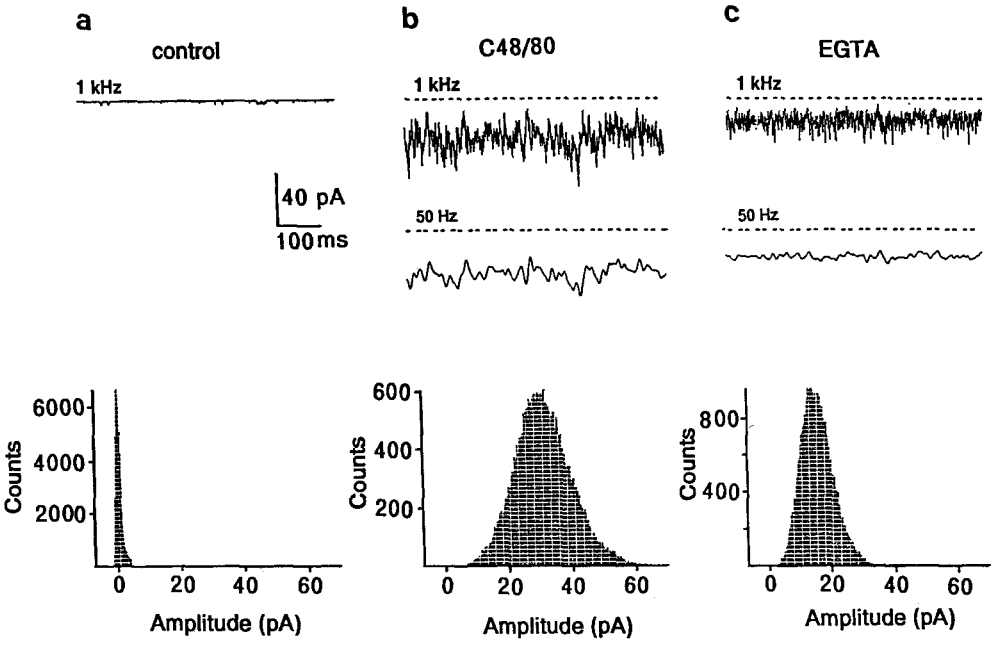

Fig. 4. Effects of EGTA on the 48/80-induced inward currents at −50 mV. Current records and amplitude histograms in control (*a*) and of the 48/80-induced currents before (*b*) and after (*c*) addition of EGTA in a cell. 48/80: 5 μg/ml. The addition of EGTA decreased the extracellular Ca^{2+} concentration from 2 mM to 1 μM. The top trace in each column shows data with low-pass filtering at 1 kHz, and the second traces in *b* and *c* show the same data after the low-pass filtering at 50 Hz. The dashed lines in *b* and *c* indicate the level of zero current. Amplitude histogram was obtained from data filtered at 1 kHz and digitized at 10 kHz. Bin width: 0.2 pA. Analysis time: 6.5 sec. The external medium contained 150 mM Na^+ and 2 mM Ca^{2+} and the internal medium contained K-glutamate.

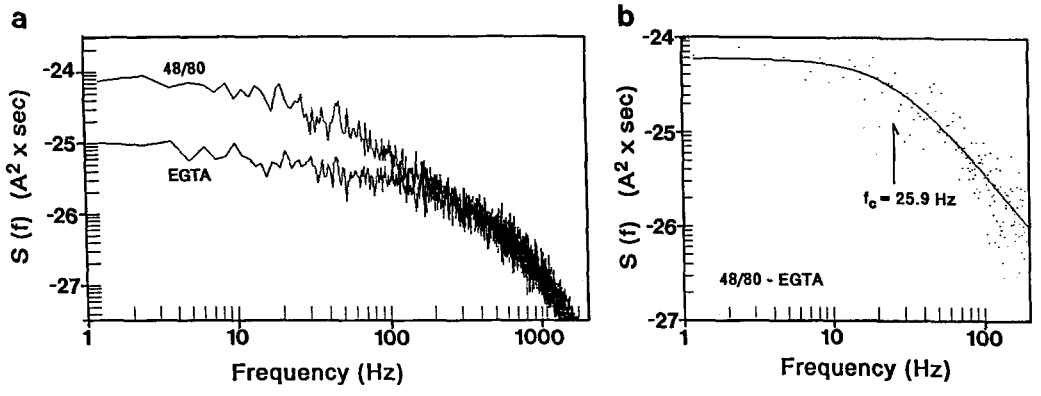

Fig. 5. The power density spectra of the 48/80-induced currents at −50 mV before and after the addition of EGTA. 48/80: 5 μg/ml. (*a*) The spectra before (48/80) and after (EGTA) addition of EGTA are superimposed. Each data point was linked. Addition of EGTA decreased the extracellular Ca^{2+} from 2 mM to about 1 μM. (*b*) The difference spectrum of the 48/80-induced currents before and after the addition of EGTA. The external medium contained 150 mM Na^+ and 2 mM Ca^{2+} and the internal medium contained K-glutamate.

ciable effects on the single channel conductance (Penner et al., 1988; Kuno et al., 1989). A clear unitary level of channel current was identified in some cells before or following stimulation in the Na^+-containing medium (Figs. 1*a*, 4*a* and 8), but seldom in the Na^+ free medium (Kuno et al., 1989). Involvement of the cation-selective channel in vigorous inward currents induced by 48/80 in the presence of Na^+ was evident in some cells, from records in which step changes corresponding to the unitary current level were occasionally identified (arrows in Fig. 8*b*). Reducing the extracellular Ca^{2+} from 2 mM to 1 μM by EGTA decreased the total current amplitude and revealed more clearly openings of

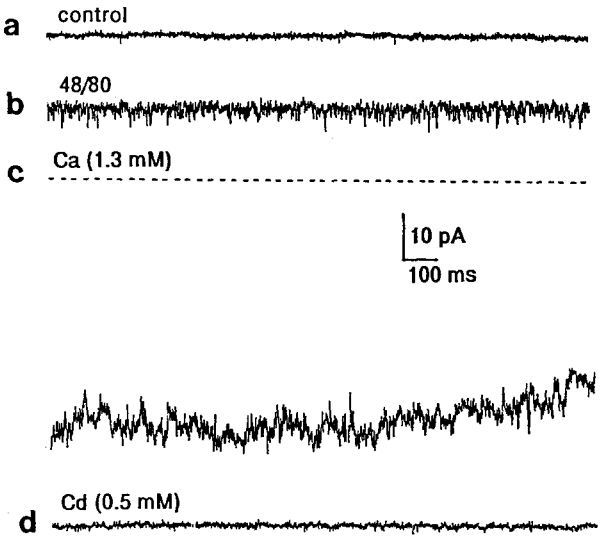

Fig. 6. Effects of reduction of extracellular Ca^{2+} prior to stimulation and re-admission of Ca^{2+} on the 48/80-induced inward currents at −50 mV. (*a*) The extracellular Ca^{2+} was reduced to 1 μM by EGTA 1 min prior to stimulation. (*b*) Addition of 48/80 in the low Ca^{2+} medium induced small rapid current fluctuations. 48/80: 5 μg/ml. (*c*) 5 min after the stimulation, 1.3 mM Ca^{2+} was added to the medium. The dashed line indicates the level of zero current. (*d*) 0.5 mM Cd eliminated the currents. The external medium contained 150 mM Na^+ and 2 mM Ca^{2+} and the internal medium contained CsCl.

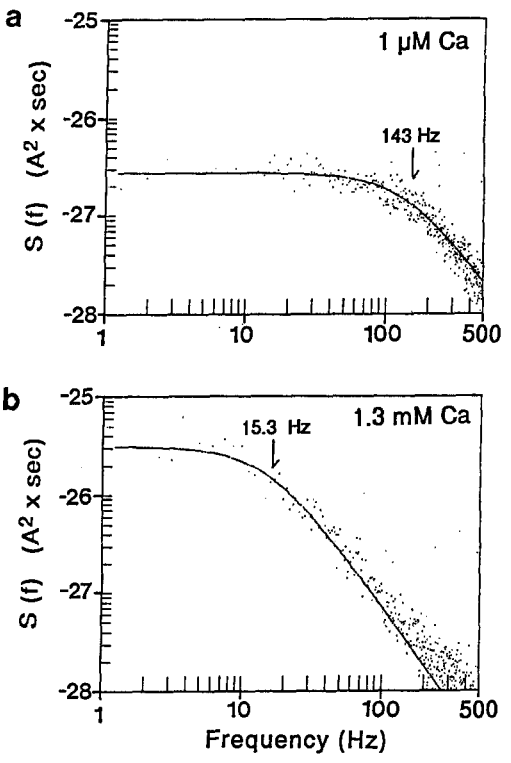

Fig. 7. (*a*) The power density spectrum of the 48/80-induced currents at −50 mV in the presence of 1 μM Ca^{2+}. (*b*) The spectrum following re-admission of Ca^{2+} at 5 min after stimulation with 48/80. The Ca^{2+} concentration was 1.3 mM. *a* and *b* were obtained from the same cell. 48/80: 5 μg/ml. The external medium contained 150 mM Na^+ and 2 mM Ca^{2+} and the internal medium contained CsCl.

the cation-selective channel (Fig. 8*c*). There was no significant change in the amplitude of the single channel current, by either 48/80 or EGTA. Thus, the reduction of the 48/80-induced currents by EGTA was unlikely due to a decrease in the single channel conductance of the cation-selective channel.

The 48/80-Induced Current Noise in the Na^+ Free Medium

When the external Na^+ was totally replaced by N-methyl-D-glucamine or choline, the mean amplitude of 48/80-induced inward currents at −50 mV in the presence of 2 mM Ca^{2+} was 7.2 ± 4.0 pA (n = 5), smaller than those in the standard Ringer solution containing Na^+ and Ca^{2+}. The power density spectrum of the 48/80-induced currents in the Na^+-free medium containing 2–5 mM Ca^{2+} was fitted by a sum of two Lorentzians in most cells (Fig. 9*a*). The mean and SD of low and high f_c in six cells were 13.7 ± 6.4 Hz and 186 ± 77 Hz. The ratio between var_L and var_H ranged from 0.75 to 5.48. The relationship between total current variance and mean current amplitude in the Na^+-free medium containing 2 mM Ca^{2+} was determined during the development of 48/80-induced currents (Fig. 9*b*). The ratio between variance and mean current amplitude at −50 mV with 2 mM Ca^{2+} was 0.17 pA in this cell and 0.25 ± 0.11 pA (n = 4) in four cells recorded with 10–20 μM DIDS, a Cl^- channel blocker, in either the external or both of the internal and external solutions. The values were less than those in the presence of Na^+.

Discussion

The present study revealed that the power density spectra of the noise of the 48/80-induced inward currents contained two components in almost all of the cells examined and that current fluctuation corresponding to the low frequency component underlies the large current noise characteristic of the 48/80-induced inward currents. The effects of extracellular Ca^{2+} on the low and high frequency components were distinct: Reduction of extracellular Ca^{2+} from 1 mM to 1 μM suppressed the low frequency component of the 48/80-induced inward currents, but not

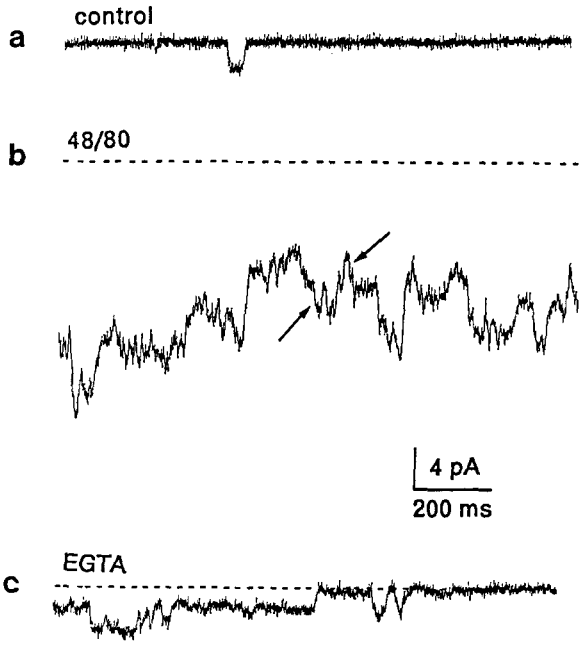

Fig. 8. The cation-selective channel activated by 48/80. The currents were recorded at −50 mV before (*a*), after stimulation with 48/80 (*b*) and addition of EGTA (*c*). The dashed line indicates the level of zero current, and arrows, unitary current levels. 48/80: 5 μg/ml. The external medium contained 150 mM Na^+ and 2 mM Ca^{2+} and the internal medium contained K-glutamate.

the high frequency component. The ratio between current variances due to the low and high frequency components was reduced by decreasing Ca^{2+}. In Na^+-free medium containing 2–5 mM Ca^{2+}, the mean current amplitude was smaller than that in the Na^+-containing standard Ringer solution, but the power density spectrum was generally composed of the low and high frequency components. These results suggest that extracellular Ca^{2+} was essential to generate the low frequency component of current fluctuation and that extracellular Na^+ augmented its amplitude.

Stimulation with 48/80 was reported to activate Cl^- channel (Penner et al., 1988; Matthews et al., 1989*b*), but contribution of the Cl^- currents to the inward current noise is likely to be negligible from the following observations: First, the f_c were not significantly different with the pipette solutions containing CsCl and K-glutamate. Second, the mean current amplitudes recorded with the two internal solutions were not significantly different as well (Kuno et al., 1989). Third, in the Na^+-free medium containing 2 mM Ca^{2+}, the single channel current estimate (the ratio between variance and mean current amplitude) was similar in the presence and absence of DIDS, a blocker for Cl^- channel in rat peritoneal mast cells. Thus, the 48/80-induced inward current noise seems to be consisted mainly of Ca^{2+} and Na^+ influx.

Other studies showed that 48/80 activated a cation-selective channel of 30–50 pS (Penner et al., 1988; Kuno et al., 1989), and the large noise was suggested to be due to the activity of the channel (Matthews et al., 1989*a*). Involvement of the channel in the vigorous inward currents was hardly discernible in many cells, but augmentation of the amplitude of the current fluctuation by extracellular Na^+ described herein supports the idea that Na^+ influx through the channel contributes to the large noise. The cation-selective channel was found to be permeable to Ca^{2+} (Penner et al., 1988); however, the Ca^{2+} influx through the channel was suggested to be negligible as deduced from the simultaneous measurement of currents and $[Ca^{2+}]_i$ by a fluorescent calcium indicator (1-2-(5′carboxyoxazol-2′-yl)6-aminobenzofuran-5-oxy-2-(2-amino-5′-methylphenoxy)ethane-N,N,N′,N′-tetraacetic acid; fura-2) in single mast cells (Matthews et al., 1989*a*). Channels other than the cation-selective channel have been suggested to be responsible for the Ca^{2+} influx (Penner et al., 1988; Kuno et al., 1989, 1990; Matthews et al., 1989*a*). The present study provides further evidence for the existene of channels contributing to the Ca^{2+} influx in the 48/80-induced inward currents: First, 48/80 induced small inward currents in the Na^+-free medium containing Ca^{2+}. The ratio between variance and mean current amplitude was small, hence the channel is likely to have a small conductance. Second, in the medium containing Na^+ and Ca^{2+} the unitary current amplitude, estimated by the parameters of the power density spectrum, varied among cells and was generally smaller than that of the cation-selective channel. If more than one class of channel is responsible for the currents, the single channel current amplitude estimated from the parameters of the current noise is an average value, weighted by both the ratios of occurrences of the channels and the conductances (Cull-Candy et al., 1988). In addition, activation of the Ca^{2+}-permeable channel in the cell-attached patches was mediated by second messengers generated via pathways sensitive to pertussis toxin, but the activation was not apparently impaired in the low Ca^{2+} medium differently from the cation-selective channel (Kuno et al., 1989, 1990). Thus, it is likely that Ca^{2+}-permeable channels with conductances smaller than that of the cation-selective channel are involved in the 48/80-induced currents.

The inhibition of the 48/80-induced inward currents in the Na^+-containing solution by lower extracellular Ca^{2+} suggested that 48/80-induced activation of the cation-selective channel was somewhat

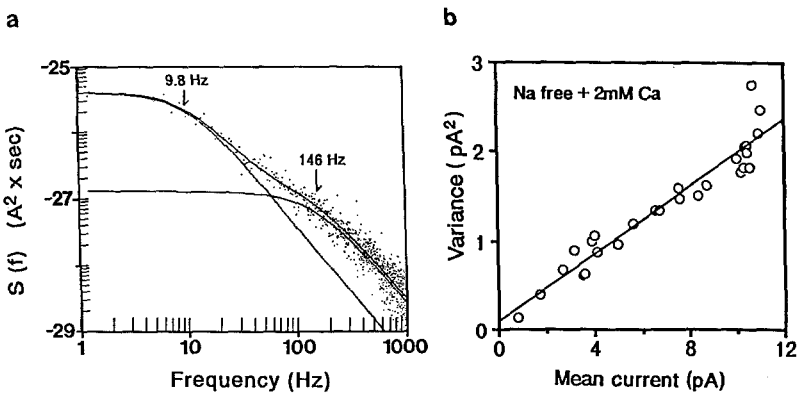

Fig. 9. The 48/80-induced currents at −50 mV in the Na^+-free medium containing 2 mM Ca^{2+}. The external Na^+ was replaced by 150 mM choline$^+$. 48/80: 5 μg/ml. (*a*) The power density spectrum. (*b*) Relation between total current variance and mean current amplitude of the induced currents during the development after onset of stimulation. Each point represents variance calculated directly from data of 1.63 sec. The line is a linear regression for data with a slope of 0.19 pA (r = 0.92). *a* and *b* were from the same cell. The internal solution contained K-glutamate.

disturbed in the low Ca^{2+} medium. Extracellular Ca^{2+} seems to act on the cation-selective channel to change the probability of the channels being open or the number of channels available and not by affecting the amplitude of the single channel current. Activation of the cation-selective channel is considered to be mediated by second messengers, but the mechanisms have not been well defined (Matthews et al., 1989*a*). Lindau and Fernandez (1986) reported that the channel was activated by dialyzing cells using pipette solution containing high Ca^{2+}, thereby suggesting that the cation-selective channel can be activated by an increase in $[Ca^{2+}]_i$, as was noted in various tissues (Colquhoun et al., 1981; Marty, Tan & Trautmann, 1984; Von-Tscharner et al., 1986; Sturgess, Hales & Ashford, 1987; Jorissen et al., 1990). In other studies, however, there was no correlation between activity of the channel and IP_3-induced Ca^{2+}-influx in single mast cells loaded with fura-2 (Penner et al., 1988; Matthews et al., 1989*a*). In addition, activation of the channel by 48/80 was attenuated by reducing external Ca^{2+} to 1 μM (Kuno et al., 1989), while the transient Ca^{2+} release from internal stores almost remained in 1 μM Ca^{2+} (White et al., 1984; Kuno et al., 1990). Extracellular Ca^{2+}-dependent events accompanied by the stimulation with 48/80 might modulate the activation of the channel, but the mechanisms remain to be solved. Thus, the external Ca^{2+} is likely to play a significant role in regulation of current activities, even if the external Ca^{2+} was not obligatory for the 48/80-induced secretion of histamine (Ennis et al., 1980).

Amount of the 48/80-induced inward currents in rat peritoneal mast cells has been reported to vary from cell to cell (Kuno et al., 1989; Matthews et al., 1989*a*). The wide range of the unitary current amplitude estimated from the parameters of spectra suggests that the heterogeneity of the 48/80-induced responses is due to variable ratio of occurrence of channels with different conductances in each cell. Candidates for channels responsible for the receptor-operated Ca^{2+} influx are suggested in mast cells (Penner et al., 1988; Kuno et al., 1989; Matthews et al., 1989*a*) and a tumor mast cell line (Mazurek et al., 1984), but it is not determined that the secretagogue-induced Ca^{2+} influx is mediated solely by one class of channel or more than two.

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