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CALCIUM DEPENDENCY OF INHIBITION BY ARACHIDONIC ACID OF COMPOUND 48/80-INDUCED HISTAMINE RELEASE FROM MAST CELLS

JUNICHI DAINAKA, ATSUSHI ICHIKAWA, MICHIKO OKADA and KENKICHI TOMITA*
Department of Health Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University, Yoshida, Sakyo-ku, Kyoto 606, Japan

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Abstract—Compound 48/80 (compd 48/80)-induced histamine secretion from rat mast cells was inhibited almost completely by pretreatment of the cells at 37° with 25 μM arachidonic acid in the presence of 1.8 mM Ca^{2+}. As the Ca^{2+} concentration was reduced below 1.8 mM, 25 μM arachidonic acid became less inhibitory and, then, progressively more stimulatory for histamine release with or without compd 48/80. No additive effect on histamine release was obtained by combining compd 48/80 and arachidonic acid. Pretreatment of mast cells with lidocaine, an inhibitor of Ca^{2+} binding to phospholipid, or with nordihydroguaiaretic acid, an inhibitor of Ca^{2+} flux and lipoxygenase, stimulated arachidonic acid-induced histamine release. Arachidonic acid also inhibited a compd 48/80-induced spike increment of intracellular $^{45}Ca^{2+}$ uptake and a decrease of total $^{45}Ca^{2+}$ uptake by $^{45}Ca^{2+}$-preloaded mast cells. Arachidonic acid and Ca^{2+} also suppressed melittin-induced histamine release and compd 48/80-induced release of radioactivity from mast cells preloaded with [^{3}H]arachidonic acid. These results suggest that exogenous arachidonic acid or its metabolite(s) may interact with membrane-associated Ca^{2+}, disturbing Ca^{2+} availability for the trigger mechanism of compd 48/80-induced histamine release or inhibiting the subsequent metabolism of arachidonic acid via the lipoxygenase pathway to form active metabolites involved in the histamine liberating mechanism.

Accumulated evidence indicates that some metabolite(s) of arachidonic acid, presumably originating from the lipoxygenase pathway, is necessary in order to trigger histamine release from mast cells [1–3] and basophilic leukemia (RBL-1) cells of the rat [4] induced by a variety of stimuli. Although exogenous arachidonic acid (AA) at high concentrations (> 50 μM) is cytotoxic and stimulatory for histamine release from these cells, preincubation of mast cells with a low concentration (1–20 μM) of AA inhibits the mediator release induced by anti-IgE [2], concanavalin A [2], or A23187 [3]. This paradoxical inhibitory action of arachidonic acid has been attributed either to membrane stabilization through increased resynthesis of phosphatidylcholine from lysophosphatidylcholine and arachidonic acid [3] or to the generation of an inhibitory metabolite(s) of arachidonic acid via the cyclooxygenase pathway [5]. However, the exact inhibitory mechanism is not entirely clear yet.

Since calcium is required as a mediator in stimulus–secretion coupling in many secretory cells [6], including mast cells [7–11], and is also involved in the regulation of metabolism of phospholipids [6], we have investigated the effect of calcium on the inhibition by arachidonic acid of histamine release from mast cells. Compound 48/80 (compd 48/80) was used as the histamine releaser because, in contrast to the antigen–antibody reaction or concanavalin A, this reagent is known to exert a histamine liberating effect on mast cells in the absence of calcium in the medium [7, 8, 12].

* Address all correspondence to: Kenkichi Tomita, Department of Health Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University, Yoshida, Sakyo-ku, Kyoto 606, Japan.

METHODS AND MATERIALS

Preparation of mast cells. Mast cells were collected from peritoneal cavity fluid of male Wistar rats weighing 300–350 g and concentrated by Ficoll density gradient centrifugation [13] to 90–93% purity, as assessed by metachromasia after staining with toluidine blue (0.1% in citrate buffer, pH 4.8). The cells were suspended in Tyrode's solution (137 mM NaCl, 2.7 mM KCl, 1.0 mM $MgCl_2$, 1.8 mM $CaCl_2$, 0.4 mM NaH_2PO_4, 11.9 mM $NaHCO_3$ and 5.6 mM glucose) containing 0.1% gelatin at pH 7.4 (Tyrode–gelatin solution). Cell number was determined with a Coulter counter (model Z, Coulter Electronics, Hialeah, FL, U.S.A.). Cell viability was determined by nigrosin exclusion [14] and also by lactate dehydrogenase release [15].

Histamine release assay. Mast cells (0.5 to 1.5 × 10^5 cells), suspended in 1 ml of Tyrode–gelatin solution, were preincubated with or without various concentrations of arachidonic acid or other substances at 37°, first for 3–10 min and then for another 10 min after the addition of compd 48/80 (0.4 μg). The reaction was terminated by cooling the mixture in an ice-bath. After centrifugation at 500 *g* for 1 min, histamine in the supernatant fraction was assayed fluorometrically by the method of Shore *et al.* [16] and expressed as a percentage of the total histamine

content of the cells, which was determined by extraction with 2.2% (final concentration) $HClO_4$.

Calcium flux assay. Mast cells (2×10^6 cells) were suspended in 0.5 ml of a 4-(2-hydroxyethyl)-1-piperazine-ethansulfonic acid (Hepes)-buffered solution which contained 10 mM Hepes-NaOH (pH 7.4), 137 mM NaCl, 4.15 mM KCl, 0.9 mM $CaCl_2$ and 1.05 mM $MgCl_2$ (Hepes medium). After the addition of tracer $^{45}CaCl_2$ (final specific activity, 0.6 Ci/mole), cell suspensions were incubated at 37° for 40 min when the specific activity of cell-associated $^{45}Ca^{2+}$ was approximately equal to that in the medium. Then 2 μl of arachidonic acid (final concentration, 25 μM), nordihydroguaiaretic acid (NDGA) (final concentration, 20 μM), or the vehicle was added directly to the radioactive incubation mixture, which was then incubated at 37° for 10 min. After a 5-sec incubation with compd 48/80 (0.4 μg) at 37°, aliquots (100 μl) of the cell suspension were filtered through HAWP 0.45 μm Millipore filters. Under the experimental conditions employed, no significant damage to cells on the filters was observed on photomicrographic examination. The filters were immediately rinsed three times either with 2 ml of cold 0.25 M sucrose to remove unbound calcium, for estimating the total $^{45}Ca^{2+}$ uptake, or with 2 ml of Ca^{2+}-free Locke solution (137 mM NaCl + 4.15 mM KCl) containing 2 mM ethylene glycol bis(β-aminoethylether)-*N*,*N*,-*N*',*N*'-tetraacetic acid (EGTA) and 3 mM $LaCl_3$ to remove surface-bound Ca^{2+}, for estimating the intracellular $^{45}Ca^{2+}$ uptake. Filters were dried, placed in counting vials, dissolved in 2 ml of methyl-cellosolve, and counted in 8 ml of 0.5% 2,5-diphenyloxazole (PPO) in toluene, as described previously [17].

Degranulation assay. Granules, extruded from mast cells, were selectively stained with ruthenium red according to Lagunoff [18]. Mast cells (*ca.* 1×10^5 cells) were suspended in 100 μl of Tyrode's solution containing 0.005% ruthenium red and incubated at 37° on a glass slide. After adding compd 48/80 (0.4 μg in 1 μl), the degranulation process was followed by color-photomicrography (about 100 cells/picture) at 5-sec intervals. Cells with more than two granules attached on the surface were counted as degranulated cells.

[^{3}H]Arachidonic acid release assay. Mast cells (11.3×10^6 cells) were suspended in RPMI 1640 medium (50 ml) containing [5, 6, 8, 9, 11, 12, 14, 15-^{3}H]arachidonic acid (25 μl). Aliquots (2.5 ml) of the cell suspension were incubated at 37° in plastic petri dishes (2.5 cm in diameter, Inter Med, Denmark) for 18 hr in a 5% CO_2-containing humidified atmosphere. During the incubation, [^{3}H]arachidonate-labeled mast cells adhered to the plastic wall. After removing the medium by suction, the labeled cells were rinsed twice with the cold Tyrode–gelatin solution (5 ml) containing 0.1% bovine serum albumin (BSA). The labeled cells were pretreated with the same solution (2 ml) containing 25 μM or no arachidonic acid for 5 min at 37° and then were incubated with compd 48/80 (0.4 μg/ml) at 37°. At various times, aliquots (200 μl) of the incubation mixture were withdrawn and centrifuged, and then the radioactivity in supernatant fractions was counted in 8 ml of 0.5% PPO in a Triton–toluene (3:1, v/v) mixture.

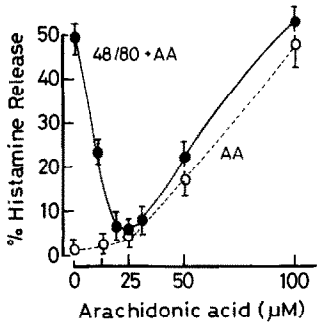

Fig. 1. Effect of arachidonic acid (AA) on histamine release induced by compd 48/80. Mast cells (1.2×10^5 cells) suspended in 1 ml of Tyrode–gelatin solution were preincubated with the indicated concentration of arachidonic acid for 5 min at 37°, and then incubated with (●) or without (○) compd 48/80 (0.4 μg/ml) for a further 10 min. Each point represents the mean ± S.D. of three determinations. Spontaneous release of histamine (2.7 ± 1.7%) was subtracted from each value. Similar results were obtained for five more experiments of the same design.

Chemicals. Chemicals used were obtained from the following sources: Arachidonic acid, compd 48/80, lidocaine, indomethacin, aspirin and nordihydroguaiaretic acid (NDGA), Sigma Chemical Co. (St. Louis, MO, U.S.A.); 3-deazaadenosine, Southern Research Institute (Birmingham, AL, U.S.A.); Hepes, EGTA, ruthenium red and quinacrine, Nakarai Pure Chemicals (Kyoto, Japan); RPMI 1640 medium, Nissui Seiyaku Co. (Tokyo, Japan); gelatin, Difco Laboratories (Detroit, MI, U.S.A.); melittin, Serva Finebiochemica GmbH & Co. (Heidelberg, West Germany); and [5, 6, 8, 9, 11, 12, 14, 15-^{3}H]arachidonic acid (80–120 Ci/mmole) and $^{45}CaCl_2$ (10–40 mCi/mg Ca), Amersham-Searle (Amersham, England). Other chemicals of reagent grade were obtained commercially.

RESULTS

Effect of arachidonic acid on compd 48/80-induced histamine release. As shown in Fig. 1, compd 48/80-induced histamine release was inhibited almost completely by preincubation of mast cells with 20–25 μM arachidonic acid in the 1.8 mM Ca^{2+}-containing medium. On the other hand, preincubation with arachidonic acid at concentrations higher than 30 μM caused progressive cell damage, as indicated by an increased percentage of nonviable cells (up to 50% at 50 μM) and by a dose-dependent histamine release, regardless of whether compd 48/80 was present in the medium or not. The simultaneous addition of arachidonic acid and compd 48/80 to the cells was less inhibitory, while the addition of arachidonic acid to compd 48/80-stimulated cells did not inhibit but, rather, augmented (by about 10%) the histamine release (data not shown).

The inhibitory effect of arachidonic acid was considerably affected by the preincubation time and temperature (Fig. 2). Mast cells, which had been pretreated with 25 μM arachidonic acid at 0° for 5 or 20 min and then washed off with cold Tyrode's solution to remove residual arachidonic acid, still showed suppressed histamine release on subsequent

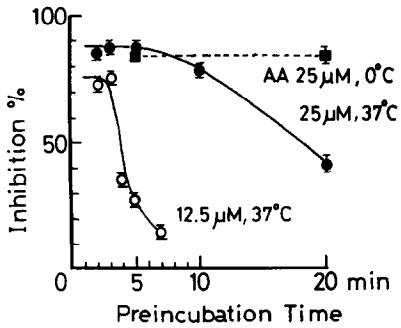

Fig 2. Effects of temperature and time on inhibition by arachidonic acid of compd 48/80-induced histamine release. Mast cells (about 0.8×10^5 cells) were preincubated with 12.5 μM (○) or 25 μM (●) arachidonic acid at 37° for the indicated times, and then treated with compd 48/80 (0.4 μg) at 37° for 10 min. Some cells were pretreated with 25 μM archidonic acid at 0° for 5 or 20 min (■), washed free of residual fatty acid at 0°, and then treated with compd 48/80 (0.4 μg). Each point represents the mean ± S.D. of three determinations. Similar results were obtained for three more experiments of the same design.

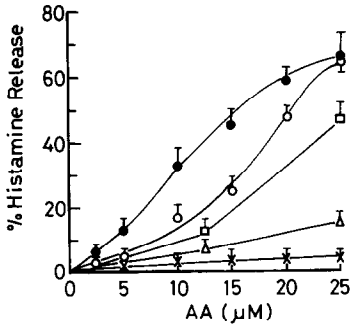

Fig. 4. Effect of Ca^{2+} on arachidonic acid-induced histamine release. Mast cells (1.1×10^5 cells) were suspended in 1 ml of Tyrode–gelatin solution, containing 1.8 mM (×), 0.9 mM (△), 0.45 mM Ca^{2+} (□) or no Ca^{2+} with (●) or without (○) 0.2 mM EGTA, and then incubated with the indicated concentration of arachidonic acid for 5 min at 37°. Each point represents the mean value ± S.D. of three determinations. Spontaneous release of histamine was subtracted from each value. Similar results were obtained for two more experiments of the same design.

exposure to compd 48/80. On the other hand, pretreatment of mast cells with 25 or 12.5 μM arachidonic acid for up to 20 min at 37° significantly reduced its inhibitory effect on compd 48/80-induced histamine release, suggesting that metabolism or degradation of this fatty acid decreases its inhibitory effect.

Effect of Ca^{2+} on inhibition by arachidonic acid of compd 48/80-induced histamine release. Compd 48/80-induced histamine release is known to be unaffected by the presence or absence of Ca^{2+} in the medium [7, 8, 12], but its inhibition by arachidonic acid required exogenous Ca^{2+} during the pretreatment as shown in Fig. 3. Mast cells pretreated with 25 μM arachidonic acid in the 1.8 mM Ca^{2+}-containing medium were protected from compd 48/80-induced histamine release by 95% in the presence of Ca^{2+} (Fig. 3, column A) and by 70% in the absence of Ca^{2+} (B) during subsequent exposure to the releaser. On the other hand, pretreatment with arachidonic acid in the absence of Ca^{2+} did not prevent compd 48/80-induced histamine release, regardless of whether Ca^{2+} was present (C) or absent (D) during exposure to the releaser. Furthermore, in the absence of Ca^{2+}, arachidonic acid (25 μM) alone stimulated histamine release (C, D), but this arachidonic acid-induced histamine release was not additively augmented by compd 48/80.

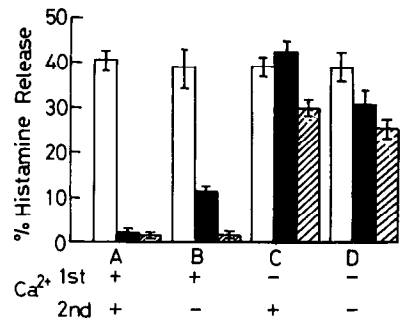

Fig. 3. Effect of exogenous Ca^{2+} on inhibition by arachidonic acid of compd 48/80-induced histamine release. Mast cells (1.2×10^5 cells) were preincubated with (closed columns) or without (open columns) 25 μM arachidonic acid in 1 ml of Tyrode-gelatin solution containing 1.8 mM Ca^{2+} (+) (A, B) or no Ca^{2+} (−) (C, D) for 5 min at 37° (first incubation). Then after centrifugation for 3 min at 0° to separate the first supernatant fraction, the cells were washed twice with 2 ml of each cold solution, resuspended in 1 ml of fresh Tyrode–gelatin solution containing 1.8 mM Ca^{2+} (+) (A, C) or no Ca^{2+} (−) (B, D) and then incubated with compd 48/80 (0.4 μg/ml) for 10 min at 37° (second incubation). Hatched columns indicate the values for the samples incubated with arachidonic acid but without compd 48/80. After centrifugation for 3 min at 0°, each supernatant fraction was combined with the corresponding one of the first incubation for the assay of histamine release. Each column represents the mean value ± S.D. of three determinations. Spontaneous release of histamine (4.5 ± 2.1%) was subtracted from each value. Similar results were obtained for three more experiments of the same design.

Histamine release by arachidonic acid was examined further by varying the concentrations of both the fatty acid and Ca^{2+} (Fig. 4). In the presence of 1.8 mM Ca^{2+}, arachidonic acid concentrations of up to 25 μM did not release significant amounts of histamine. However, as the Ca^{2+} concentration was decreased below 1.8 mM, 2.5 to 25 μM arachiconic acid progressively stimulated histamine release. In the absence of added Ca^{2+} or the deprivation of Ca^{2+} by incubating the cells with EGTA, arachidonic acid as low as 2.5 μM caused detectable histamine release.

As shown in Fig. 5, the maximum inhibitory effect of 25 μM arachidonic acid on histamine release induced by compd 48/80 (0.4 μg/ml) was obtained in the presence of a 1.8 mM, or higher, concentration of Ca^{2+}. However, when the Ca^{2+} concentration was decreased progressively below 1.8 mM, 25 μM arachidonic acid not only inhibited the compd 48/80-induced histamine release less, but eventually was also progressively more stimulatory for the histamine release.

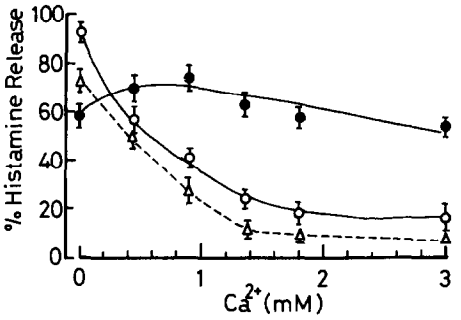

Fig. 5. Effect of Ca^{2+} on histamine released by arachidonic acid and/or compd 48/80. Mast cells (5×10^4 cells) were suspended in 1 ml of Tyrode–gelatin solution containing various concentrations of Ca^{2+} (0–3 mM), preincubated with 25 μM arachidonic acid for 5 min at 37°, and then challenged with compd 48/80 (0.4 μg/40 μl) for 10 min. Key: compd 48/80 alone (●——●), arachidonic acid alone (△——△), and arachidonic acid plus compd 48/80 (○——○). Each value represents the mean ± S.D. of three determinations. Similar results were obtained for two more experiments of the same design.

Table 1. Effects of various divalent cations on inhibition by arachidonic acid of histamine release induced by compd 48/80*

	Histamine release		
Divalent cation	Compd 48/80 (%)	Compd 48/80 + arachidonic acid (%)	Inhibition (%)
Ca^{2+}	34.5 ± 0.3	0.8 ± 0.1	97
Co^{2+}	8.1 ± 0.1	0	100
Ni^{2+}	13.7 ± 1.3	1.6 ± 0.1	98
Mn^{2+}	27.1 ± 1.1	18.2 ± 0.8	33
Sr^{2+}	51.5 ± 0.8	41.6 ± 0.5	19

* Mast cells (0.8×10^5 cells/ml) suspended in Ca^{2+}-free Tyrode–gelatin solution supplemented with 1.8 mM of one of the divalent cations, were preincubated with or without 25 μM arachidonic acid for 5 min at 37°. The cells were then challenged with 0.4 μg of compd 48/80 for 10 min at 37°. Each value represents the mean ± S.D. of three determinations. Similar results were obtained for two more experiments of the same design.

The preference for Ca^{2+}, over the other divalent cations, of arachidonic acid inhibitory activity toward compd 48/80-induced histamine release is shown in Table 1. Pretreatment of mast cells with arachidonic acid in Tyrode's solution that contained 1.8 mM Co^{2+} or Ni^{2+}, instead of Ca^{2+}, effectively suppressed compd 48/80-induced histamine release, but these two cations also significantly inhibited compd 48/80-induced histamine release by themselves. In the presence of Mn^{2+} or Sr^{2+}, compd 48/80-induced histamine release was not suppressed effectively by pretreatment with arachidonic acid in comparison to pretreatment in the presence of Ca^{2+}.

Effects of various compounds on histamine release. As shown in Table 2, pretreatment of mast cells with lidocaine, an inhibitor of calcium binding to phospholipid, did not affect compd 48/80-induced histamine release at 1 mM, reduced it at 10 mM, and stimulated arachidonic acid-induced histamine release at both 1 and 10 mM. As a consequence, lidocaine appeared to abolish the inhibition by arachidonic acid of compd 48/80-induced histamine release. However, in spite of the stimulation of arachidonic acid-induced histamine release in the presence of lidocaine, no additive stimulatory effect was obtained by the combination of compd 48/80 and arachidonic acid, suggesting that the inhibitory effect of arachidonic acid may not be reduced significantly. Nordihydroguaiaretic acid (NDGA), an inhibitor of Ca^{2+} flux in neutrophils [19] and mast cells (see Table 3), inhibited the histamine release induced by compd 48/80, but stimulated that induced by arachidonic acid. The histamine release stimulated by arachidonic acid and NDGA was not further augmented by compd 48/80. Quinacrine, an inhibitor of phospholipase A_2, inhibited compd 48/80-induced histamine release, but it did not affect the treatment with arachidonic acid. Ethacrynic acid (0.1 mM), an inhibitor of Ca^{2+}-dependent ATPase, 3-deazaadenosine (1 mM), an inhibitor of methyltransferase,

Table 2. Effects of various agents on inhibition by arachidonic acid of compd 48/80-induced histamine release*

Pretreatment	No addition	Compd 48/80 (0.4 μg/ml)	Arachidonic acid (25 μM)	Arachidonic acid + compd 48/80
		(% of total cellular histamine)		
None (control)	0	39.3 ± 1.5	4.5 ± 0.2	8.2 ± 0.3
Lidocaine 1 mM	2.4 ± 0.5	45.5 ± 0.1	21.4 ± 0.5	30.9 ± 2.1
10 mM	5.2 ± 0.6	9.5 ± 0.3	95.0 ± 2.0	84.5 ± 2.0
NDGA 0.02 mM	0.8 ± 0.5	2.6 ± 1.3	46.6 ± 3.4	40.3 ± 3.8
Quinacrine 2 mM	2.2 ± 0.2	8.1 ± 0.7	5.0 ± 0.3	9.6 ± 0.1

* Mast cells (0.9×10^5 cells/ml) were preincubated for 5 min at 37° in the presence or absence of various compounds and then were incubated with or without 25 μM arachidonic acid for a further 5 min at 37°. The cells were then challenged with 0.4 μg of compd 48/80 for 10 min at 37°. Each value represents the mean ± S.D. of three determinations. Spontaneous release (1.5 ± 0.2%) was subtracted from each value. Similar results were obtained for three more experiments of the same design.

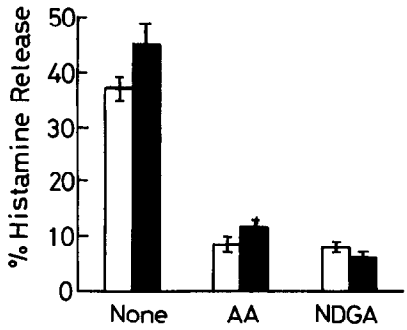

Fig. 6. Effects of arachidonic acid and nordihydroguaiaretic acid on melittin-induced histamine release. Mast cells (0.8×10^5 cells) were preincubated with 25 μM arachidonic acid or 20 μM NDGA in 1 ml of Tyrode–gelatin solution for 5 min at 37° and then incubated with 0.2 μg of melittin for a further 5 min at 37°. Then one-half of each sample was incubated with 0.4 μg of compd 48/80 for another 10 min at 37° (closed columns), and the other half with the vehicle (open columns). Each column represents the mean value ±S.D. of three determinations. Spontaneous release of histamine (3.7%) was subtracted from each value. Similar results were obtained for three more experiments of the same design.

and aspirin and indomethacin (1 μM–1 mM), both inhibitors of cyclooxygenase, did not inhibit compd 48/80-induced histamine release, with or without arachidonic acid (data not shown).

Effects of arachidonic and nordihydroguaiaretic acids on melittin-induced histamine release. Melittin, a bee venom, is known to be an activator of phospholipase A_2 [20], as well as a potent histamine releaser [21]. Melittin-induced histamine release was not augmented by further addition of compd 48/80 (Fig. 6). Furthermore, both arachidonic acid and NDGA inhibited histamine release induced not only by compd 48/80 (Table 2) but also by melittin, with or without compd 48/80. These results suggest that both compd 48/80 and melittin stimulate similar metabolic pathway(s) of arachidonic acid required for histamine release, and that the exogenous arachidonic acid may inhibit the activation of phospholipase A_2 or the subsequent conversion of arachidonic acid to active metabolite(s) required for histamine release via the lipoxygenase pathway.

Table 4. Effect of arachidonic acid on [^{3}H]arachidonic acid release from mast cells*

	[^{3}H]Arachidonic acid released (cpm/10^7 cells)	
	Time after compd 48/80 addition	
	2.5 sec	10 sec
None	2340 ± 154	4990 ± 205
Arachidonic acid	<100	2460 ± 180

* Experimental procedures are described under Methods and Materials. Each value represents the mean ± S.D. of three determinations. Similar results were obtained for two more experiments of the same design.

Effect of arachidonic acid on calcium uptake of mast cells. As shown in Table 3, intracellular $^{45}Ca^{2+}$ amounted to about one-tenth of the total $^{45}Ca^{2+}$ of mast cells equilibrated with $^{45}Ca^{2+}$ (0.9 mM) at 37° for 30 min. Immediately (3–10 sec) after the addition of compd 48/80, a spike increment of intracellular $^{45}Ca^{2+}$ and a decrease of the total $^{45}Ca^{2+}$ were observed with a maximum response at 5 sec. During this pulse period, no significant granule extrusion occurred. Percentages of degranulated cells were 10.6 ± 1.2% at 5 sec and 12.5 ± 1.4% at 10 sec for compd 48/80-treated cells and 9.7 ± 0.6% at both 5 and 10 sec for untreated cells. Arachidonic acid added to mast cells during $^{45}Ca^{2+}$-equilibration slightly increased the total $^{45}Ca^{2+}$ and prevented both the compd 48/80-induced decrease of total $^{45}Ca^{2+}$ and spike increment of intracellular $^{45}Ca^{2+}$. On the other hand, NDGA inhibited the compd 48/80-induced increase of intracellular $^{45}Ca^{2+}$ uptake but did not prevent the decrease of total $^{45}Ca^{2+}$. Furthermore, the intracellular $^{45}Ca^{2+}$ uptake was not elevated by stimulating the cells with compd 48/80 at 0°, suggesting that the entry of $^{45}Ca^{2+}$ into the intracellular compartment requires energy metabolism.

Effect of arachidonic acid on [^{3}H]arachidonic acid release activity in mast cells. Arachidonic acid (25 μM) markedly inhibited compd 48/80-induced

Table 3. Effects of arachidonic acid, nordihydroguaiaretic acid and compd 48/80 on $^{45}Ca^{2+}$ uptake of mast cells*

		Intracellular $^{45}Ca^{2+}$ (nmoles/10^6 cells)			Total $^{45}Ca^{2+}$ (nmoles/10^6 cells)		
	Temperature	No addition	Arachidonic acid	Nordihydro-guaiaretic acid	No addition	Arachidonic acid	Nordihydro-guaiaretic acid
None	37°	3.62 ± 0.25	4.31 ± 0.23	4.05 ± 0.50	34.2 ± 1.9	38.3 ± 2.8	32.1 ± 3.0
	0°	3.35 ± 0.32	4.08 ± 0.35		30.8 ± 5.2	35.2 ± 2.3	
Compd 48/80	37°	8.12 ± 0.45	5.14 ± 0.37	4.30 ± 0.50	26.3 ± 1.1	35.8 ± 1.5	25.3 ± 2.5
	0°	3.66 ± 0.53	4.19 ± 0.25		29.3 ± 2.9	34.6 ± 3.5	

* Mast cells (2×10^6 cells/0.5 ml) that had been preloaded with 0.9 mM $^{45}CaCl_2$ (0.6 Ci/mole) for 40 min were preincubated with 25 μM arachidonic acid or 20 μM nordihydroguaiaretic acid in Hepes medium for 10 min and then were incubated with 0.4 μg of compd 48/80. After a 5-sec incubation at 37°, aliquots (100 μl) of the cell suspensions were filtered through HAWP 0.45 μm Millipore filters. The filters were immediately rinsed three times with 2 ml of either 0.25 M sucrose (for total $^{45}Ca^{2+}$) or Ca^{2+}-free Locke solution containing 2 mM EGTA and 3 mM $LaCl_3$ (for intracellular $^{45}Ca^{2+}$), dried, and then counted. Each value represents the mean ± S.D. of four samples.

release of radioactivity from [^{3}H]arachidonic acid-prelabeled mast cells (Table 4).

DISCUSSION

The present results show that pretreatment of mast cells with low concentrations (~25 μM) of arachidonic acid inhibited the histamine release induced by compd 48/80, as reported for the mediator release induced by other agents such as antigen-antibody, concanavalin A and A23187 [2, 3], and that effective inhibition by arachidonic acid required an appropriate concentration of extracellular Ca^{2+} during the pretreatment but not during the subsequent exposure to compd 48/80.

Although compd 48/80-induced histamine release is independent of extracellular Ca^{2+}, it still shows a general pattern of Ca^{2+}-dependent exocytosis; Douglas and his coworkers [7, 8] demonstrated that little or no histamine is released by compd 48/80 from Ca^{2+}-depleted mast cells in a Ca^{2+}-free environment, and that the responsiveness to compd 48/80 is restored immediately upon addition of Ca^{2+}. In the present study, we observed a spike increase of intracellular $^{45}Ca^{2+}$ and a decrease of total $^{45}Ca^{2+}$ within a few seconds after exposure of $^{45}Ca^{2+}$-equilibrated mast cells to compd 48/80 without any significant granular extrusion (Table 3). These results suggest that compd 48/80 is able to utilize membrane-associated Ca^{2+} effectively for mediator release by acutely mobilizing it for transference into the cells to cause a small increase in the size of the exchangeable intracellular Ca^{2+} compartment and by partially discharging it from the cells. Apparently, the resulting transient increase of intracellular Ca^{2+} causes the histamine release.

The prevention by exogenous arachidonic acid of compd 48/80-induced mobilization of calcium in $^{45}Ca^{2+}$-preloaded mast cells (Table 3) suggests a possible formation of an electrostatically stable complex between membrane-associated $^{45}Ca^{2+}$ and arachidonic acid or its metabolite(s). The following results seem to further support this hypothesis: (1) arachidonic acid increased the total $^{45}Ca^{2+}$ uptake in both compd 48/80-stimulated and -unstimulated cells at 0°, but not the intracellular $^{45}Ca^{2+}$ uptake (Table 3); (2) an appropriate concentration of Ca^{2+} was required for effective inhibition by arachidonic acid of compd 48/80-induced histamine release, regardless of whether the cells were pretreated with arachidonic acid at 37° or even at 0° (Figs. 2, 3, and 5); (3) Ca^{2+}, but not other divalent cations had to be present during preincubation with arachidonic acid prior to stimulation by compd 48/80 (Table 1 and Fig. 3); (4) in a Ca^{2+}-depleted medium, as low as 2.5 μM arachidonic acid caused detectable histamine release (Fig. 4); and (5) both lidocaine, a suppressor of cellular Ca^{2+} binding, and NDGA, an inhibitor of Ca^{2+} flux, stimulated arachidonic acid-induced histamine release, while they inhibited compd 48/80-induced histamine release.

Goth and Knoohuizen [1] reported that, as in the case of histamine release induced by anti-IgE and other stimuli [2–4], compd 48/80-induced histamine release was also inhibited by eicosa-5,8,11,14,-tetraynoic acid, a blocker of both the lipoxygenase and cyclooxygenase pathways of arachidonic acid metabolism. We observed that, in addition to arachidonic acid, NDGA, a potent inhibitor of Ca^{2+} flux in neutrophils [19, 22] and also of lipoxygenase [23], inhibited the histamine release induced by compd 48/80 (Table 2) and by melittin, a potent phospholipase A_2 activator [19], as well (Fig. 6). In addition, no additive effect was obtained with melittin and compd 48/80 (Fig. 6) or with arachidonic acid and compd 48/80 (Table 1) on histamine release. Quinacrine, an inhibitor of phospholipase activity, inhibited both compd 48/80- and arachidonic acid-induced histamine release (Table 3). These results suggest that compd 48/80 stimulates the conversion of arachidonic acid to active metabolite(s) required for histamine release via the lipoxygenase pathway, in a manner similar to that by which endogenous arachidonic acid generated by the action of melittin is converted. Furthermore, the inhibition by arachidonic acid of melittin-induced histamine release (Fig. 6) and of compd 48/80-induced [^{3}H]arachidonic acid release from mast cells (Table 4) suggests that exogenous arachidonic acid may inhibit its own generation by phospholipase A_2, as suggested for concanavalin A [24]- and A23187 [3]-stimulated mast cells, or by the phospholipase C-diacylglycerol lipase reaction, as reported for compd 48/80-stimulated mast cells [25], and may also inhibit its own metabolism via the lipoxygenase pathway. The origin of free arachidonic acid involved in histamine release needs further investigation.

However, the activities of phospholipase A_2 from rabbit platelets [26] and phospholipase C from rat liver [27] have been reported to be dependent on the Ca^{2+} concentration. A possible requirement of Ca^{2+} for lipoxygenase activity has also been reported for basophil leukemia cells [28]. Thus, a disturbance of Ca^{2+} availability for these enzymes by arachidonic acid *in vitro* may inhibit their activities in generating the metabolite(s) of arachidonic acid that is required to trigger the histamine release mechanism.

Based on the observation that indomethacin abolishes the inhibition by arachidonic acid of histamine release induced by cross-linking of surface IgE molecules of mast cells, Marquardt *et al.* [5] suggested that certain cyclooxygenase products of arachidonic acid inhibit the releasing mechanism. However, in the present study, indomethacin or aspirin (1 μM ~ 1 mM) did not block the inhibitory action of arachidonic acid on compd 48/80-induced histamine release, suggesting no involvement of cyclooxygenase products of arachidonic acid. The reason for the different response to indomethacin or aspirin is not clear, except for the difference in the stimulants used. However, the metabolic pathway of exogenous arachidonic acid in mast cells has not been fully elucidated yet. This certainly requires further investigation.

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