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Inhibition of Histamine Release and Ionophore-Induced Calcium Flux in Rat Mast Cells by Lidocaine and Chlorpromazine[1]

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Abstract

We studied the effects of lidocaine (L) and chlorpromazine (C), two compounds known to affect the binding of calcium to cell membranes, on histamine release and ^{45}calcium uptake by purified mast cells upon challenge with the ionophore A23,187 or with compound 48/80. At low concentrations L and C inhibited the Ca^{++} flux as well as histamine release while higher concentrations caused enhancement in this function. Evidence was obtained that L 10^{-4} *M* may displace Ca^{++} from the cell membranes.

Introduction

Rat mast cells, which require calcium for the secretory release of mediators, are a current and excellent model to study this secretory process. Local anaesthetics such as lidocaine, procaine and tetracaine as well as the sedative and antiemetic chlorpromazine have been shown to exert their pharmacological effect by binding calcium at membrane surfaces of cells [1, 2]. Calcium may be prevented from entering the cell or actually be displaced from membrane receptors by these agents [2], by intercalating into the lipid-protein mosaic of the membrane.

The secretion of histamine from mast cells stimulated by antigen-antibody reactions is dependent on calcium [3, 4]; secretion is a result of the influx of calcium [4]. This event is thought to occur by a change in membrane permeability to calcium, and ion-specific ionophores that selectively increase the permeability of cell membranes to calcium have been used as valuable tools to study the mechanism [5–7]. The calcium ionophore A23,187 (Lilly) appears to bypass immunospecific ligand-receptor interactions at the cell membrane both in lymphocytes [8, 17] and in mast cells [9], therefore it can be used as a convenient pharmacological probe into the role of calcium and inhibitors on the release mechanism.

Local anesthetics and membrane active, molecules that become incorporated into biological membranes have been shown to affect many cellular functions dependent upon calcium permeability, and may modulate Ca^{++} flux as well as release. In cellular immunity, effector function of cytotoxic lymphocytes, mitogen-induced lymphocyte aggregation and blast transformation could be inhibited by lidocaine and chlorpromazine [10, 11]. In immediate hypersensitivity reactions, lidocaine, tetracaine and chlorpromazine have been shown to inhibit both antigen-antibody and 48/80-induced release of mediator of anaphylaxis [12–14, 24]. This report deals with attempts to use an ionophore and 48/80, both of which cause histamine release by increasing mast cell calcium levels, as pharmacological tools to define the role of the membrane surface active agents, chlorpromazine and lidocaine in the inhibition of histamine release and $^{45}Ca^{++}$ flux. It differs from previous reports [12, 13], because it expands the profile of L and C and shows a dose response for inhibition curve in both L and C which shows that ^{45}Ca flux and histamine release are inhibited by similar concentrations of L and C.

Materials and methods

Mast cell histamine release

Mast cells were obtained by peritoneal lavage from 250-g Sprague Dawley (Upj-TUC SD/Spf) rats asphyxiated with CO_2, with 15 ml of cold Hanks BSS containing 50 units

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of heparin USP per ml. The cells were washed twice in Hanks BSS and resuspended in Tyrodes buffer, pH 7.4 (g/l: NaCl, 8.0; KCl, 0.20; $CaCl_2 \cdot 2H_2O$, 0.20; $MgCl_2 \cdot 6H_2O$, 0.10; NaH_2PO_4, 0.050; $NaHCO_3$, 1.0; glucose, 1.0) containing 2% normal heat inactivated (56°C × 2 h) rat serum. The cells were purified by layering on Ficoll gradients made up at 20, 30, 40, 50% in the above Tyrodes buffer but without rat serum. Centrifugation at 90 × *g* for 15 min followed by recovery of the 40% layer of Ficoll gave cells 85–90% in purity with respect to mast cells. One to two million mast cells were incubated at 37°C in 500 μl Tyrodes buffer containing A23,187 for 20 min. A23,187 (a gift from Eli Lilly Co., Indianapolis, IN) was made up at 10 mg/ml in 100% DMSO and diluted in Tyrodes buffer before use. The final concentration of DMSO never exceeded 0.01% and did not affect release or inhibition. In the same way 48/80 (Burroughs Wellcome Co., Tuckahoe, NY) 0.1–0.5 μg/ml was added to purified or nonpurified mast cells suspended in Tyrodes + 2% rat serum.

After incubation, cells were removed by centrifugation at 600 × *g* for 10 min. The protein was precipitated from the supernates by making them 0.4 *M* with respect to $HClO_4$, and histamine was determined in the acid supernatants and in the boiled extracts of the sedimented cells by spectrofluorometric assay [15], as modified by SIRAGANIAN [16] using an automated Technicon instrument. Histamine release was expressed as a percentage of total histamine available and was corrected for endogenous (control) release. When lidocaine or chlorpromazine was added, they were made up in water and added to the cells 5 min prior to the ionophore or 48/80.

^{45}Calcium flux assay

$^{45}CaCl_2$ (New England Nuclear), having a specific activity of 16.9 mCi/mg, was added to Ficoll-purified mast cells at the rate of 10 μCi per 10^6 cells suspended in Tyrodes buffer. 500-μl aliquots of cells were removed from the 37°C incubation and filtered over 25-mm 8-μ Millipore filters. The filters were washed three times with 5-ml volumes of phosphate-buffered saline (PBS), pH 7.4 (g/l: NaCl, 4.27; KH_2PO_4, 2.25; Na_2HPO_4, 7.87), dissolved in 0.1 ml glacial acetic acid, and counted in 15 ml Diotol solution on a Packard model 3375 Scintillation Spectrometer with a counting efficiency of >98%. Net ^{45}Ca uptake was calculated by correcting for background binding to filters and cells at zero time. A23,187 was a gift from Eli Lilly, Indianapolis, IN.

^{45}Calcium displacement assay

Purified mast cells (60 × 10^6) were incubated for 20 min with 1.0 μg/ml ionophore and 600 μCi $^{45}CaCl_2$ in 45 ml Tyrodes buffer. The cells were centrifuged (600 × *g* for 10 min) and washed twice in Tyrodes + 2% rat serum. The cell pellet was split and to one-half was added 10^{-4} *M* lidocaine and to the control, saline. Aliquots of these cells (6 × 10^5) were then incubated at 37°C for additional time increments then processed as above on Millipore filters.

Results

When lidocaine was added to cells before A23,187 or 48/80, both the ionophore-induced ^{45}calcium flux and histamine release were altered as follows: (1) inhibition of histamine release induced by 48/80 was maximal at 10^{-4} *M* with a return to near maximal 48/80 release at 10^{-2} *M* lidocaine, and (2) there appeared to be a correlation between the inhibition of ionophore-induced $^{45}Ca^{++}$ flux by lidocaine and the inhibition of histamine release (Figs. 1, 2). The maximal inhibition of $^{45}Ca^{++}$ flux was also at 10^{-4} *M* lidocaine with an enhancement of up to 200% $^{45}Ca^{++}$ flux when 10^{-2} *M* lidocaine was used, this was not evident in enhanced histamine release.

When chlorpromazine was used in similar studies, its maximal concentration for inhibition of ionophore-induced $^{45}Ca^{++}$ flux was 10^{-5} *M* with little effect at lower concentrations (Figs. 1, 2). This increase in inhibitory activity is presumably due to the fact that when measurements were made of the solubility and binding of lidocaine and chlorpromazine into cell membranes, lidocaine was bound at 3.5 and chlorpromazine at 1400 molecules per unit weight of cellular membranes [2].

Therefore, in two systems of release, histamine and ionophore calcium, which are presumed to be different in mechanism [9], lidocaine, and to a lesser extent, chlorpromazine in-

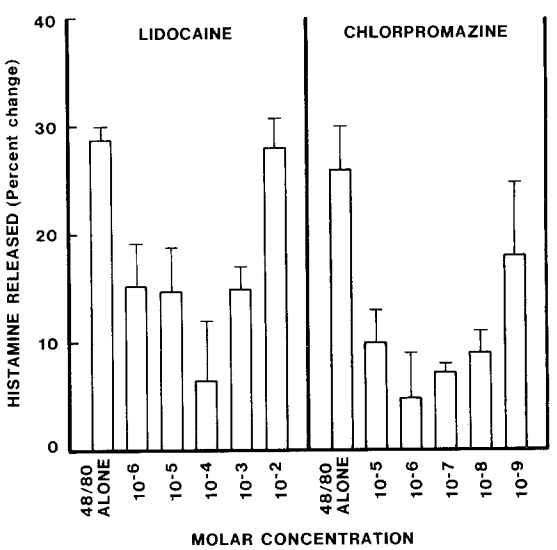

Figure 1
The influence of lidocaine and chlorpromazine on 48/80 (0.20 μg/ml) induced histamine release. The cells were incubated at 25°C with the local anesthetics (10^{-2}–10^{-4} *M*) for 2 min prior to 48/80. Mean ± S.E.M. of five experiments and percent change were based on comparison to 48/80 alone and boiled cell complete release. Histamine was assayed using an automated Technicon instrument with five replicates at each variable.

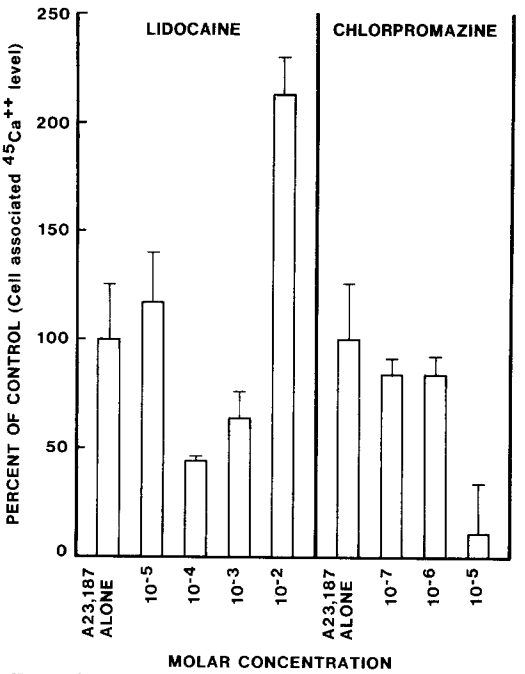

Figure 2
The influence of lidocaine and chlorpromazine on ionophore A23,187 (0.1 μg/ml) induced ^{45}calcium flux in Ficoll purified rat mast cells. The purified cells were incubated at 37°C for 20 min with (10^{-2}–10^{-7} *M*) anesthetic in cells which contained 10 μCi $^{45}CaCl_2$ per 10^6 cells. The cells were aliquoted onto Millipore filters, washed, and counted in Diotol solution. Mean ± S.E.M. of five experiments with five replicates for each variable. ^{45}Ca was counted with a counting efficacy of >98%.

hibited histamine release in a bell-shaped dose response curve with enhanced release often being seen at extremely high concentrations of the two agents.

Attempts were made to study the nature of the inhibition by local anesthetics of the release mechanism. Is it by displacement of membrane-bound calcium, or is it a chelation reaction? Neutral anesthetics displace membrane-bound calcium as one way they inhibit uptake, for example, in the sarcolemma of cells [2]. Studies in mast cells were undertaken to attempt to displace bound $^{45}Ca^{++}$ with lidocaine from cells which had been labelled with $^{45}Ca^{++}$ in the presence of ionophore then washed to remove external $^{45}Ca^{++}$ to see if mast cells are similar to muscle cells. Table 1 shows the results of such an experiment. After labelling and washing to remove any free ionophore and extracellular calcium, 10^{-4} *M* lidocaine, shown above to be the most active concentration for inhibiting histamine release, also displaced calcium from prelabelled cells. The displacement did not appear to be time-related in kinetics, but the loss compared to control cells was significant ($p < 0.05$). Over the time span of incubation there was undoubtedly loss and reuptake in control cells and lidocaine-treated cells, but the loss was greater in the presence of lidocaine. Equilibrium between intracellular and extracellular calcium was undoubtedly not reached since the ionophore itself is removed by continued washing [2] and would also pull out bound calcium.

Discussion

Changes in the intracellular concentration of calcium ions provide the control for many physiologic processes, including secretion [19]. The Ficoll-purified, isolated rat mast cell is a

Table 1
Displacement of ^{45}calcium with lidocaine from mast cells labelled in the presence of ionophore.[a]

Time after lidocaine addition (min)	Net ^{45}calcium c.p.m.[b]		
	Control cells ave. c.p.m. (±SD)	Cells + lidocaine 10^{-4} *M*	
		Ave. c.p.m. (±SD)	C.p.m. loss due to lidocaine
2	3148 (±849)	1670 (±241)	1478
5	2987 (±615)	1823 (±304)	1164
20	1875 (±702)	1557 (±201)	1318
30	2626 (±246)	1826 (±103)	800
60	3528 (±165)	2053 (±103)	1475

[a] 60×10^6 mast cells were labelled by exposure to 1.0 μg/ml A23,187 and 600 μCi $^{45}Ca^{++}$ for 20 min. Washed cells were resuspended as shown in Methods and incubation at 37°C was continued.
[b] The averages are from quintuplicate assays for each time point and variable.

useful model for studying the mechanism of histamine secretion, because the degree of secretory activity is readily assessed by assay of the mediators released, and the external environment of the cell can be readily changed and controlled. The use of two interesting pharmacological agents, ionophores and local anesthetics, have made it possible to apply these agents in studies that attempt to define the role of calcium in histamine release in mast cell. It has been shown [20] that ionophores cause histamine release and increased calcium flux and further, that antigen-antibody reactions at the surface of mast cells caused a similar phenomenon. Local anesthetics prevent calcium uptake into membranes as their primary site of action [2]. When all three components, ionophores, local anesthetics and mast cells were combined in experiments designed to study the mechanism of release, the following things were found: (1) at physiological concentrations both lidocaine and chlorpromazine were capable of preventing histamine release if they were present in incubations with mast cells and given sufficient time to become integrated into the cell membrane before the releaser was added. The difference in the concentrations needed for maximum inhibition, 10^{-4} *M* for lidocaine and 10^{-6} *M* for chlorpromazine can readily be explained on the ability of the agents to be incorporated into membranes. In a recent literature report, saturation kinetic studies showed that lidocaine is bound at 3.5 molecules and chlorpromazine at 1400 molecules per unit weight of membrane [2]. The enhancement seen with lidocaine at high concentrations is undoubtedly due to the increased membrane fluidity induced by these drugs [2]. Membrane-membrane fusion has been described [2] under high anesthetic concentrations. An enhancement of secretory noncytotoxic release could follow from such a fusion [2, 14]; (2) a more quantitative measure of cellular regulation of secretion, namely calcium flux, showed a correlation between the increase of cell-associated calcium concentration and the subsequent release of histamine. This influx or uptake was also inhibited by lidocaine and chlorpromazine at concentrations which inhibit a histamine release. At concentrations where enhanced histamine secretion was seen, more calcium was found inside the mast cell, for example, at 10^{-2} *M* lidocaine; (3) the specificity of the local anesthetics for inhibition of mediator release appears to be broad because both ionophore and 48/80-induced release were inhibited by these agents. There are some striking differences and similarities in the mechanism of release by these two agents. 48/80 causes an initial drop in internal cAMP and calcium levels, like antigen-antibody reactions, but then the level returns to normal and release proceeds [9, 22, 24]. Ionophore simply bypasses most preliminary steps to release and has its effect at the calcium trigger step [9]. It can therefore be postulated that the local anesthetics exert their protective effect against membrane perturbation by a non-specific change in membrane permeability to calcium as a consequence of their solubility in the biomembrane; (4) the bell-shaped dose response curve is reminiscent of that seen for cromolyn sodium (DSCG) in the inhibition of histamine release induced by 48/80, antigen-antibody reactions, or ionophore [6, 14, 18]. Actual increases in histamine release could often be seen at both ends of the dose response for DSCG [6, 19, 22, 23]. Evidence presented here with these agents supports the hypothesis that cromolyn sodium and possibly local anesthetics inhibit release by preventing calcium influx.

One could speculate, therefore, that a critical step in the cellular economy of calcium is blocked by local anesthetics, a step that causes less calcium to be available for use in release of mediators. This anesthetic concentration, critical for blockade of release, could also lead to enhanced release if it were allowed to go extremely high (in anesthetic excess 10^{-2} *M*) as in membrane-membrane fusion and increased fluidity. The displacement of membrane-bound calcium, by neutral anesthetics, could also contribute to the calcium economy of the cell.

The use of the interesting compounds like ionophores and local anesthetics makes continuing studies into the mechanism of release feasible.

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