Rat mast cells permeabilized with Sendai virus secrete histamine in response to Ca^{2+} buffered in the micromolar range

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In the presence of low extracellular Ca^{2+} , Sendai virus generates permeability lesions in the membrane of rat mast cells. This causes leakage of intracellular phosphorylated metabolites and lactate dehydrogenase; it also permits uptake of normally impermeant aqueous solutes such as the complex of Ca^{2+} with *N*-hydroxyethylethylenediamine-triacetic acid. We have used this system to buffer the concentration of cytosol Ca^{2+} in the micromolar range and thus to cause release of the contents of the secretory granules such as histamine and β -*N*-acetylglucosaminidase. We argue that such release occurs by a normal exocytotic secretory mechanism for the following reasons. (1) While leakage of cytosol components is progressively inhibited by Ca^{2+} in the range 1–10 μM , release of histamine is dependent on Ca^{2+} in this range. Higher concentrations of Ca^{2+} are inhibitory to both permeabilization and histamine release. (2) While leakage of phosphorylated metabolites is enhanced in metabolically inhibited cells and the leakage of lactate dehydrogenase is insensitive to metabolic inhibition, the release of histamine and β -*N*-acetylglucosaminidase is strictly dependent on an intact metabolic process.

Histamine secretion from rat mast cells occurs when there is an increase in the cytosol concentration of Ca^{2+} (Gomperts, 1977). This can be accomplished in a number of different ways. Thus the normal physiological stimulus is the crosslinking of cell surface IgE by specific antigen (Mota, 1957), and under these circumstances secretion is dependent on extracellular Ca^{2+} in the range 0.2–1.0 mM (Foreman & Mongar, 1972). The Ca^{2+} ionophores [A23187 (Foreman *et al.*, 1973), ionomycin (Bennett *et al.*, 1979) and 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane-4,6-dione ('fod') (Gomperts *et al.*, 1981)] also cause secretion and as the dependence on extracellular Ca^{2+} varies inversely with ionophore concentration it follows that it is the Ca^{2+} salts of the ionophores which are the agonistic form (Bennett *et al.*, 1979). In the case of ionomycin it was shown that secretion occurs as the concentration of the Ca^{2+} salt is varied in the range 1×10^{-7} – 8×10^{-7} M. However, neither these experiments, nor any experience with the receptor-directed ligands, can be used to determine the concentrations of cytosol Ca^{2+} which are compatible with the

secretory process. For this purpose it is necessary either to introduce sensing dyes for Ca^{2+} into the cytosol (in which case the dyes themselves might interfere with secretion; Tsien, 1981) or to manipulate cytosol Ca^{2+} at levels which cause secretion. We have reported experiments of the latter type by the use of mast cells treated with ATP^{4-} (Cockcroft & Gomperts, 1979*a,b*, 1980); this has the effect of generating lesions through which normally impermeant aqueous solutes of M_r up to at least 400 can pass. We found that secretion occurs as the cytosol concentration of Ca^{2+} is varied in the range 1×10^{-6} – 10×10^{-6} M (Bennett *et al.*, 1981*a*).

Another agent which causes permeability changes of cell membranes is haemolytic Sendai virus. Thus it causes depolarization of the membrane within seconds of its application to cells (Impraim *et al.*, 1980; Forda *et al.*, 1982) and this is followed by leakage of intracellular metabolites (Pasternak & Micklem, 1973; Impraim *et al.*, 1980) and uptake of normally impermeant water-soluble fluorescent dyes of M_r up to 800 (Wyke *et al.*, 1980). In a number of respects the effects on membrane permeability of ATP^{4-} on mast cells and of Sendai virus (which affects a wide range of cell types: Poste & Pasternak, 1978; Pasternak & Micklem, 1981) are similar. There is a previous report showing that

Abbreviations used: Ig, immunoglobulin; HEDTA, *N*-hydroxyethylethylenediaminetriacetic acid; h.a.u., haemagglutinin units.

Sendai virus can induce histamine secretion from mast cells in a manner resembling the IgE-directed agonists (Sugiyama, 1977). We now report that Sendai virus can permeabilize mast cell membranes and so permit the entry of Ca^{2+} and Ca^{2+} buffers directly into the cytosol, which causes secretion.

Materials and methods

Throughout this work, the concentration of Ca^{2+} was controlled by the use of the Ca^{2+} chelate HEDTA used as a buffer. Stock buffered Ca^{2+} solutions were prepared at 30 mM-HEDTA (i.e. $10 \times$ final concentration) and the concentration of free Ca^{2+} under the experimental conditions was determined with a Ca^{2+} -sensing electrode (Orion Research). This involved diluting the Ca^{2+} buffer stocks at 37°C into a buffered salt solution, pH 7.6, which comprised 137 mM-NaCl, 2.7 mM-KCl, 20 mM-4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (Hepes), 5.6 mM-glucose and bovine serum albumin ($1 \text{ mg} \cdot \text{ml}^{-1}$).

The experiments described concern the measurement of secretory products (mainly histamine, but occasionally β -N-acetylglucosaminidase) and cytosol components (^{32}P -labelled metabolites and lactate dehydrogenase) from mast cells treated in the presence of low (micromolar) concentrations of Ca^{2+} and Sendai virus.

In an experiment in which all these factors were to be determined, mixed peritoneal cells from Sprague-Dawley rats were incubated with [^{32}P]P_i ($100 \mu\text{Ci} \cdot \text{ml}^{-1}$) for 1 h in the buffered salt solution, which was supplemented with 0.5 mM-MgCl₂ and 0.9 mM-CaCl₂. The cells were then washed by centrifugation, and the mast cells purified to better than 90% homogeneity by centrifugation through 35% (w/v) bovine serum albumin (Path-O-Cyte 4, Miles Laboratories) (Lagunoff, 1975). The cells were then washed twice by centrifugation and resuspension in the buffered salt solution, and filtered through nylon gauze to remove clumps. The cells (typically suspended at a density equivalent to $0.25 \mu\text{mol}$ of total histamine $\cdot \text{ml}^{-1}$) were then added to equal volumes of salt solutions containing suspended virus and buffered Ca^{2+} at 37°C as detailed in the text.

A representative sample of cells was lysed by addition of Triton X-100 (1%) and then diluted by the addition of 5 vol. of an ice-cold quenching solution, pH 7, which comprised 0.15 M-NaCl, 10 mM-potassium phosphate and 0.1 mM-NADH. This preparation was used for the determination of total cellular quantities of ^{32}P -labelled metabolites, lactate dehydrogenase, histamine and β -N-acetylglucosaminidase. Experience showed that it was important to determine total lactate dehydrogenase without delay, since the activity declined with time,

even when stored at ice temperature and in the presence of proteinase inhibitors such as Trasylol or diisopropylphosphofluoridate; exteriorized lactate dehydrogenase from mast cells treated with Sendai virus was stable for at least 5 h. For this reason measurement of lactate dehydrogenase from detergent-lysed cells was set in train with the least possible delay (i.e. during the time the cells were being incubated with the virus).

Incubations were terminated by addition of 5 vol. of quenching solution and the cells were sedimented by centrifugation (at 400 g for 5 min) at 4°C . The supernatants were then subjected to centrifugation (at 42000 g for 30 min) to sediment suspended virus particles. The purpose of this was twofold: the absorbance traces obtained during measurement of lactate dehydrogenase became less subject to noise, and the virus preparation itself contained enzyme activity which was reactive with the substrate used for determination of β -N-acetylglucosaminidase.

In the experiment illustrated in Fig. 2, half of the cells (labelled with ^{32}P as described above) were incubated for 20 min in the absence of glucose before addition of $5 \mu\text{M}$ -antimycin A; after a further 10 min, the cells were centrifuged to remove accumulated extracellular ^{32}P . After resuspension in the presence of antimycin A (no glucose) the incubations with virus were commenced as quickly as possible. Control cells were treated similarly, but in the presence of glucose and in the absence of the inhibitor.

In the experiments illustrated in Figs. 5 and 6, the cells were treated initially in the presence of $0.5 \mu\text{M}$ - Ca^{2+} with the virus at 10 times its final concentration. At the times stated, the cells and virus were diluted 10-fold into solutions containing Ca^{2+} buffered at the stated concentrations.

The amount of histamine released was determined fluorimetrically as previously described (Cockcroft & Gomperts, 1979b). β -N-Acetylglucosaminidase was determined fluorimetrically using 4-methylumbelliferyl- β -D-glucopyranoside (Koch-Light Laboratories) as substrate, essentially according to the method of Barrett (1972). Lactate dehydrogenase was determined by measuring the initial rate of decline of absorbance at 340 nm in the solutions at 37°C following addition of 5 mM-potassium pyruvate. Release of ^{32}P -labelled metabolites (presumptively P_i, nucleotides and sugar phosphates; Cockcroft & Gomperts, 1979a) was determined by Čerenkov or liquid-scintillation counting methods.

Sendai virus was grown in the allantoic cavity of 10-day-old chick embryos for 3 days. The allantoic fluid was centrifuged at 1000 g for 10 min and the supernatant centrifuged at 40000 g for 20 min to sediment the virus. Virus amounts are expressed as h.a.u. (Davenport *et al.*, 1960). With the exception

of timecourse experiments, all Figures present means of duplicate determinations. The results presented are fully representative of experiments that were carried out on at least three occasions.

Results

Fig. 1 illustrates the dependence on Ca^{2+} of histamine release and leakage of ^{32}P -labelled metabolites from mast cells treated with different concentrations of Sendai virus. Whereas the effect of Ca^{2+} on metabolite leakage is exclusively inhibitory, release of histamine is both dependent on Ca^{2+} and then inhibited as the concentration of Ca^{2+} is elevated above about $10\ \mu\text{M}$. The lowest concentration of Ca^{2+} required to cause release of histamine from virus treated cells is about $1\ \mu\text{M}$. This concentration is similar to that needed to elicit secretion from mast cells rendered permeable to low-molecular-weight, normally impermeant, solutes due to treatment with ATP^{4-} (Bennett *et al.*, 1981a). It is also in accord with the affinity for Ca^{2+} of intracellular Ca^{2+} -binding proteins [such as calmodulin (Means *et al.*, 1982), gelsolin (Yin & Stossel, 1980) and villin (Glenney *et al.*, 1980)] which are thought to be involved in secretory and related motile processes. The finding of a requirement for Ca^{2+} in the micromolar range thus gives credence to the idea that histamine release from virus-permeabilized mast cells occurs by a secretory rather than a lytic mechanism. Other experiments reported below will reveal the distinction between these two mechanisms.

Earlier experience has shown that virus-induced permeabilization of Lettre cells can be inhibited in a

complex manner by Ca^{2+} (Pasternak & Micklem, 1974; Impraime *et al.*, 1979, 1980). There are several variables involved, and these include the ratio virus:cells, as well as a number of less tangible factors such as the history of the virus (e.g. the period of storage, the number of freeze-thaw cycles, etc.). The inhibitory effect of Ca^{2+} on virus-treated mast cells is clearly exerted on both metabolite leakage and on release of histamine, and, as shown in Fig. 1, the inhibitory effect of Ca^{2+} declines as the amount of virus is increased. This is in accordance with previous findings with Lettre cells. We find it reasonable to conclude that both metabolite leakage and secretion are initiated by a common interaction of the virus with the cells.

While the dependence on Ca^{2+} in the micromolar range is suggestive of a secretory (or exocytotic) mechanism for the release of histamine from virus-treated mast cells, we find that manipulations involving the virus are also accompanied by release of lactate dehydrogenase. This is indicative of cell lysis. An experiment illustrating the dependence on virus concentration of the release of histamine, lactate dehydrogenase and ^{32}P -labelled metabolites is recorded in Table 1. In this and other similar experiments, it is quite clear that leakage of lactate dehydrogenase becomes increased over control levels at the lowest concentrations of the virus required to cause detectable release of histamine. Similar to the leakage of ^{32}P -labelled metabolites both here and in the experiment of Fig. 1, the effect of Ca^{2+} on leakage of lactate dehydrogenase is solely inhibitory, in clear distinction to its effect on release of histamine.

In order to define further the mechanisms in-

Fig. 1. Dependence on Ca^{2+} of histamine release and leakage of ^{32}P -labelled metabolites from mast cells treated with different concentrations of virus
●, Histamine release; ○, leakage of ^{32}P -labelled metabolites.

involved, we have tested the effect of metabolic inhibition on the release of both secretory and cytosol components from virus-treated mast cells. The results of a representative experiment are shown in Fig. 2. We found that whereas the effect of inhibition is to prevent the release of histamine (and of β -N-acetylglucosaminidase), the release of cytosol components is either unaffected (lactate dehydrogenase; results not shown) or, as in the case of 32 P-labelled metabolites, enhanced. In the latter case, the increase probably arises from the effects of metabolic

inhibition on the distribution of the label between the various metabolites involved; thus, there would be a tendency for the concentration of the more permeant intracellular P_i to increase at the expense of nucleotides and glycolytic intermediates. In the poisoned cells, the protective effect of Ca^{2+} on virus-induced leakage of 32 P-labelled metabolites was completely lost (Fig. 2); this is in agreement with experience with Lettre cells (K. J. Micklem, unpublished work). In contrast with the effect on low-molecular-weight solutes, the protection by Ca^{2+} on leakage of lactate dehydrogenase from virus-treated and poisoned cells was only slightly reduced (by approx. 30%; results not shown).

In respect of its sensitivity to metabolic inhibition, the release of histamine and β -N-acetylglucosaminidase resembles secretion due to IgE-directed agonists and the Ca^{2+} ionophores, which is known to occur by an exocytotic mechanism (Lawson *et al.*, 1977).

In considering the interaction of the virus with mast cells we have been aware that the mass-action assumptions normally applied with soluble agonists might be invalid. The extent of interaction might be more appropriately governed by the ratio of virus particles to the number of cells, and virus-induced effects would be expected to increase both with increase in the amount of virus (as in the experiments of Figs. 1 and 2) and with a decrease in the cell concentration. We have some indications that this might indeed be the case. Fig. 3 illustrates the results of an experiment in which we measured the dependence on Ca^{2+} of both histamine secretion and lactate dehydrogenase leakage through a series

Table 1. *Dependence on virus concentration of histamine release and leakage of 32 P-labelled metabolites and lactate dehydrogenase in the presence of low levels of Ca^{2+}*

32 P-labelled mast cells were incubated at a concentration equivalent to $0.5 \mu\text{mol}$ of histamine $\cdot \text{ml}^{-1}$ for 20 min together with Sendai virus and buffered Ca^{2+} . Results have been corrected for loss of markers in the absence of virus. Determinations were carried out in quadruplicate.

Virus (h.a.u. $\cdot \text{ml}^{-1}$)	Ca^{2+} (μM)	Histamine release (%)	Leakage of 32 P-labelled metabolites (%)	Leakage of lactate dehydro- genase (%)
1.65	0.5	2	21 ± 1	2 ± 0.6
	10.5	0	9 ± 4	1 ± 0.3
15	0.5	2 ± 0	51 ± 4	9 ± 0.2
	10.5	12 ± 1.2	21 ± 0.2	4 ± 0.5
133	0.5	3 ± 0.2	65 ± 2.3	20 ± 0.3
	10.5	47 ± 1.1	41 ± 3.8	14 ± 0.6

Fig. 2. *Effect of metabolic inhibition on release of histamine and 32 P-labelled metabolites at different concentrations of virus and Ca^{2+}*

○, ●, Histamine release; △, ▲, leakage of 32 P-labelled metabolites. Solid symbols indicate normal incubations carried out in the presence of 5.6 mM-glucose; open symbols indicate incubations carried out with inhibited cells. Metabolic inhibition was achieved by preincubation (of half the cells) for 20 min in the absence of glucose, followed by 10 min with $5 \mu\text{M}$ -antimycin A. The cells were then washed by centrifugation (to remove accumulated extracellular ^{32}P) and resuspended in glucose-free buffer containing $5 \mu\text{M}$ -antimycin. Control cells were similarly preincubated and washed, but were maintained in glucose-containing (no antimycin) buffer throughout.

of three 5-fold dilutions of the cells at two virus concentrations.

The most evident manifestation of the ratio effect is the increased degree of leakage of lactate dehydrogenase (at $0.5 \mu\text{M-Ca}^{2+}$) as the cells are

Fig. 3. Effect of cell density on the dependence on Ca^{2+} of histamine release and leakage of lactate dehydrogenase at 4 (○) and 40 (●) h.a.u. $\cdot \text{ml}^{-1}$ (a) 10^6 cells $\cdot \text{ml}^{-1}$; (b) 0.2×10^6 cells $\cdot \text{ml}^{-1}$; (c) 0.04×10^6 cells $\cdot \text{ml}^{-1}$.

diluted out in the presence of the higher concentration of the virus. There was also a tendency for the extent of histamine release to decline with increasing cell dilution at the higher virus concentration, presumably due to increasing cell damage. At the lower concentration of the virus, the extent of histamine release increased as the cell number was reduced, possibly as the ratio virus:cells tended towards an optimal level; in this case we would anticipate that inhibition would have occurred had the cells been diluted out still further.

Fig. 4 illustrates a series of time-course experiments for ^{32}P -labelled metabolite leakage and histamine secretion at three virus concentrations, with Ca^{2+} buffered at 0.4 and $6.4 \mu\text{M}$. As in previous experiments, the effect of Ca^{2+} is to reduce the extent of ^{32}P -labelled metabolite leakage, while except at the highest virus concentration used in this experiment, secretion is almost totally dependent on Ca^{2+} . We found that while the extent of secretion varies with the amount of virus in this range, the extent of leakage of ^{32}P -labelled metabolites (at 10 and 20 min) was not so affected. The rate of emergence of ^{32}P -labelled metabolites is certainly increased at the higher virus concentration and so it is probable that the access of the Ca^{2+} -buffer to the cell interior is similarly accelerated. According to our earlier experience with Ca^{2+} ionophores, this would be sufficient to explain the enhanced degree of secretion (Bennett *et al.*, 1979).

Fig. 4. Time course of histamine release and ^{32}P -labelled metabolite leakage from mast cells treated with Sendai virus at (a) 45, (b) 90 and (c) 180 h.a.u. $\cdot \text{ml}^{-1}$ Solid symbols indicate $0.4 \mu\text{M-Ca}^{2+}$; open symbols indicate $6.4 \mu\text{M-Ca}^{2+}$.

Fig. 5. Time course of histamine release from cells treated with Sendai virus initially in the presence of low ($0.5 \mu\text{M}$) Ca^{2+} and then subjected to $6.4 \mu\text{M}$ - Ca^{2+} after delays of (a) 0, (b) 0.5, (c) 1 and (d) 2 min

In this Figure the extent of release is normalized against the extent of release which occurred at 12 min [(a) 16.5%, (b) 24%, (c) 24% and (d) 11.5%]. In this experiment the cells were treated with the virus initially at $100 \text{ h.a.u.} \cdot \text{ml}^{-1}$ at the times stated, the cells and the virus were diluted 10-fold into solutions containing $6.4 \mu\text{M}$ - Ca^{2+} so that final virus concentration was $10 \text{ h.a.u.} \cdot \text{ml}^{-1}$.

Fig. 6. Dependence on Ca^{2+} of histamine secretion from mast cells treated with Sendai virus initially in the presence of low ($0.5 \mu\text{M}$) Ca^{2+} and then subjected to step changes as indicated after delays of (a) 0, (b) 0.5, (c) 1, (d) 2, (e) 2.5 and (f) 4 min

The cells were treated as described in the legend to Fig. 5; in this experiment, the final concentration of virus was $12.5 \text{ h.a.u.} \cdot \text{ml}^{-1}$.

The appearance of secreted histamine occurs after a delay of up to 2 min depending on the amount of virus used. The experiment illustrated in Fig. 5 was designed in order to find out whether this delay could be ascribed to events occurring before or after the virus-treated cells become sensitive to the effects of extracellular Ca^{2+} . The time course of secretion was followed either from cells treated in the normal way with virus and Ca^{2+} -buffer simultaneously, or from cells which were treated with the virus in the presence of $0.5 \mu\text{M}$ - Ca^{2+} for 0.5, 1 or 2 min before increasing the $[\text{Ca}^{2+}]$ to $6.4 \mu\text{M}$. We found that secretion commenced immediately when Ca^{2+} was added to cells pretreated with the virus, and further, that secretion under these conditions was considerably enhanced. The delay normally observed is thus due to rate limitation in the interaction of the virus with the cells which leads to permeabilization, and not to subsequent events.

The experiment illustrated in Fig. 6 presents a

systematic statement of the enhancement of secretion which occurs when Ca^{2+} is added to cells pretreated with the virus, and it also offers a possible explanation. While confirming that for cells treated normally the effect of increasing Ca^{2+} (in the range 19 – $54 \mu\text{M}$) is to inhibit and ultimately to prevent secretion we now find that for cells pretreated with the virus, the degree of inhibition by the higher concentrations of Ca^{2+} is much reduced. Earlier, in reference to Figs. 2 and 3, we drew attention to the inhibitory effect of Ca^{2+} both on secretion and on the leakage of ^{32}P -labelled metabolites from virus-treated cells; in that case, the effect of Ca^{2+} was to inhibit the formation of the lesions through which metabolites leak out, and through which Ca^{2+} buffers can gain access to the cytosol. In the present case of cells pretreated with the virus, the lesions were induced in the presence of $0.5 \mu\text{M}$ - Ca^{2+} , thus producing maximal permeability. The subsequent step change in free Ca^{2+} increases cytosol Ca^{2+} but

Table 2. *Effect of flavonoids and their glycosides on Sendai-virus-induced histamine secretion*

The cells were treated with Sendai virus (100 h.a.u. · ml⁻¹) at 37°C in the presence of Ca²⁺ (0.5 μM) and inhibitors as indicated for 30 s. The concentration of Ca²⁺ was then brought to 15 μM and the incubation continued for a further 10 min. In this experiment the secretion due to the virus in the absence of inhibitory substances was 26 ± 0.25% with 15 μM-Ca²⁺, and 2.5 ± 0.075% with 0.5 μM-Ca²⁺ (± S.E.M., *n* = 8). Taken as a group, release of histamine with 0.5 μM-Ca²⁺ in the presence of the inhibitors was 2.9 ± 0.15% (*n* = 8).

	Concn. (μM)	Inhibition (%)
Aglycones		
Quercetin	30	26
	100	49
Phloretin	30	42
	100	51
Glycosides		
Rutin	30	26
	100	42
Phloridzin	30	12
	100	52

does not inhibit the virus-induced permeability, suggesting that the inhibitory effect of Ca²⁺ is exerted only at the stage of formation of the virus-induced lesions.

A number of agents and manipulations known to modulate or inhibit histamine secretion from mast cells stimulated by IgE-crosslinking were tested. Phosphatidylserine (20 μM), which is known to enhance secretion due to IgE-directed agonists (Goth *et al.*, 1971), was without effect in the virus system. Table 2 records the effects of some flavonoids on virus-mediated secretion. These compounds were tested in the knowledge that while the aglycones (quercetin, phloretin) are inhibitors of IgE-mediated secretion, their glycosides (rutin, phloridzin) (Fewtrell & Gomperts, 1977; Bennett *et al.*, 1981*b*) are without effect. In virus-mediated secretion (in which we treated the cells at 0.5 μM-Ca²⁺ together with the virus and the inhibitors for 30 s before imposing a step change in the concentration of Ca²⁺), we now found that aglycones and glycosides have similar activity as inhibitors. This confirms the suggestion that the site of inhibition for compounds of this class lies within the cells (Bennett *et al.*, 1981*b*).

The following substances, tested under the same conditions, were without effect on virus-induced histamine secretion: cyclic AMP (10–1000 μM), cyclic GMP (10 μM), isobutylmethylxanthine (1 mM), suramin (100 μM) and cytochalasin B (5 μM). Inhibitors of virus-induced secretion include the tranquillizer pimozide (1 μM), possibly through

action on calmodulin, nordihydroguaiaretic acid (100 μM) which is thought to affect cells through inhibitory effects on phospholipases, and adenosine 5'-[βγ-imido]triphosphate (5 mM) possibly acting as a competitor for intracellular ATP.

We also tested the effect of preincubating cells at 47°C for 20 min prior to stimulating with the virus; this treatment is known to prevent secretion due to the IgE-directed ligands and compound 48/80 (Mongar & Schild, 1957). In spite of increasing the extent of leakage of lactate dehydrogenase both in the presence and absence of virus, virus-dependent histamine release was prevented.

Discussion

Treatment of mast cells with Sendai virus increases the permeability of the plasma membrane. This results in the leakage of ³²P-labelled metabolites from the cells and the entry into the cytosol of normally impermeant aqueous solutes such as the complex of Ca²⁺ with HEDTA. Such manipulations allow the direct control of cytosol Ca²⁺ at defined levels in the micromolar range. When the free Ca²⁺ in the cytosol rises to 1 μM or above, this treatment causes the release of histamine and other components of the secretory granules (we measured β-N-acetylglucosaminidase) from the cells. In addition, Ca²⁺ at and above these levels progressively inhibits the induction of the virally mediated permeability change, and consequently the extent of leakage of cytosol components falls. When this becomes limiting (above approx. 5 μM-Ca²⁺) the extent of histamine release also declines.

When extensive permeabilization of cells by Sendai virus occurs, they undergo lysis and release high-molecular-weight compounds such as lactate dehydrogenase. At higher concentrations of Ca²⁺ (>100 μM) this effect is insignificant (Pasternak & Micklem, 1974) but under the conditions of low Ca²⁺ described here, the release of the cytosol enzyme approaches that of histamine (Table 1). As histamine release from mast cells damaged with detergents (e.g. decylamine; Bloom & Haegermark, 1967) or some pharmacological agents such as *Physalia* (Portuguese Man O'War) venom (Flowers & Hessinger, 1981) is accompanied by loss of lactate dehydrogenase and lytic mechanisms have been inferred, it is of the greatest importance to consider carefully whether the appearance of extracellular histamine from virus-treated cells occurs as a consequence of lysis, or by the mechanism of exocytosis.

For the following reasons, we firmly believe that an exocytotic (secretory) process is involved.

(1) The Ca²⁺-concentration dependence of the release of histamine is similar to that previously demonstrated in mast cells permeabilized by ATP⁴⁻

(Bennett *et al.*, 1981a), and in platelets (Knight & Scrutton, 1980, 1982) and adrenal chromaffin cells (Baker & Knight, 1981) permeabilized by dielectric breakdown. In contrast, lytic release of histamine due to decylamine or *Physalia* venom is insensitive to Ca^{2+} .

(2) Concentrations of Ca^{2+} above $1\mu\text{M}$ (but below $20\mu\text{M}$) have an inverse effect on release of histamine and lactate dehydrogenase.

(3) While release of lactate dehydrogenase is insensitive to metabolic inhibition, release of histamine is prevented. Such inhibitors are without effect on lytic release of histamine from cells treated with decylamine or *Physalia* venom.

(4) At supraoptimal virus:cell ratios, where lactate dehydrogenase leakage is maximal, there is a reduction in histamine release indicative of cell damage.

The effect of altering the virus:cell ratio on the leakage of ^{32}P -labelled metabolites and lactate dehydrogenase is entirely consistent with the known effects of Sendai virus on cells (Pasternak & Micklem, 1974). Also the lag in permeabilization demonstrated in Fig. 5 is characteristic of the action of Sendai virus (Pasternak & Micklem, 1973) and reflects a co-operative effect in the induction of permeabilization (K. J. Micklem, unpublished work).

The release of lactate dehydrogenase is probably a consequence of the increase in permeation by low-molecular-weight compounds. This has been inferred in red cells where the haemolytic action of the virus is due to colloid osmotic swelling following an increase in permeability to low-molecular-weight compounds, particularly Na^+ and K^+ (Impraim *et al.*, 1980).

In an earlier report concerning the effect of Sendai virus on mast cells some stress was laid on the resemblance between this form of activation and that due to the IgE-directed ligands (Sugiyama, 1977). Thus, concentrations of Ca^{2+} in the millimolar range were required and the extent of secretion was enhanced by the presence of exogenous phosphatidylserine. The results of our experiments indicate an interaction quite unlike that of the IgE-directed ligands; thus, Ca^{2+} is required in the micromolar range of concentrations, and we could find no effect of phosphatidylserine. Rather, the effect of the virus is seen to resemble those manipulations which cause cell permeabilization to normally impermeant aqueous solutes. The concentration of the virus used in the earlier work extended from 500 up to $8000\text{h.a.u.}\cdot\text{ml}^{-1}$, which is the range commonly used to cause cell-cell fusion. One would predict that this concentration would be required to overcome the inhibitory effect of Ca^{2+} at millimolar concentrations on the virus-induced permeability change. In the present work we are using low concentrations of the virus to admit Ca^{2+}

chelates and ions and consequently buffering cytosol Ca^{2+} at predetermined concentrations. Using Sendai virus to permeabilize cells to unbuffered Ca^{2+} may well give qualitatively different results. Preliminary work with anterior pituitary cells has shown differing secretory responses to Sendai virus at millimolar or micromolar free Ca^{2+} (Gillies *et al.*, 1981), presumably in an analogous way.

An alternative possibility is that the virus applied at high concentration has the effect of a lectin which might be capable of crosslinking IgE, or its receptors (Bachi *et al.*, 1973). In this case, dependence on Ca^{2+} at millimolar concentrations and potentiation by phosphatidylserine would be expected.

Conclusion

The increased permeability of plasma membranes following the treatment of mast cells by Sendai virus can be used to introduce calcium buffers into the cytosol. This stimulates secretion by a normal exocytotic mechanism.

The method has the advantages of ease and simplicity with the disadvantage of high background of lysis as a consequence of the permeability change, but should be applicable to a wide range of cell types.

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