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Apomorphine-induced inhibition of histamine release in rat peritoneal mast cells

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1 The apomorphine-induced inhibition of histamine release in rat peritoneal mast cells was studied by means of secretagogues stimulating different pathways of mast cell activation.

2 Apomorphine inhibited the mast cell response to all releasing agents (lysophosphatidylserine plus nerve growth factor, compound 48/80, substance P, ATP, tetradecanoylphorbolacetate, melittin). The IC_{50} ranged from 4 μM to 24 μM at concentrations of secretagogues releasing 30–50% of mast cell histamine. However, the potency of the drug decreased at higher segretagogue concentrations.

3 Mast cells, pretreated with apomorphine and washed, released little histamine upon stimulation. The secretory response could be partially restored on increasing the concentration of secretagogues.

4 The results suggest that apomorphine affects a regulatory step controlling the terminal sequence of mast cell secretory activity. As indicated by the reduced potency of the drug, the control by the apomorphine-sensitive reaction loses efficiency under conditions of massive histamine release.

Introduction

Micromolar concentrations of apomorphine inhibit histamine release in rodent mast cells (Mietto *et al.*, 1984). The failure to reproduce this effect with equivalent concentrations of dopamine or to prevent the inhibition by the addition of antidopamine drugs shows that the action of apomorphine cannot be taken as an indication of inhibitory dopamine receptors in mast cells. The biochemical changes occurring during mast cell degranulation include mobilization of extracellular or intracellular calcium (Pearce, 1982), enhanced phospholipid metabolism (Kennerly *et al.*, 1979), cyclic AMP production and activation of protein kinases (Winslow & Austen, 1984; Nishizuka, 1984). These events may be investigated by the use of proper mast cell activators. In this study, selected secretagogues were used to gain more information on the inhibitory effect induced by apomorphine in rat mast cells.

Methods

Peritoneal mast cells were collected from male albino rats (Sprague Dawley) of 300–350 g and purified on 35% bovine serum albumin (Boarato *et al.*, 1984). Trypan blue exclusion test revealed between 96 and 99% viability. The histamine content was, on average, 15 μg per 10^6 cells (histamine base).

Incubation of mast cells

Histamine release was determined in 1.0 ml of a medium containing (mM): Na_2HPO_4 4, KH_2PO_4 2.7, NaCl 150, KCl 2.7, $CaCl_2$ 1.0, glucose 10 and 1 mg ml^{-1} bovine serum albumin (pH 7.2). Mast cells 250×10^3 (3.8 μg histamine base) were added and preincubated for 10 min at 37°C with apomorphine before the addition of the secretagogue. The incubation was then continued for an additional 15 min at 37°C (30 min when the secretagogue was tetradecanoylphorpholacetate (TPA)) and stopped by centrifugation of the mast cells. When the experiments had to be performed in the absence of extracellular calcium, the cation was omitted in the final wash of the mast cells and in the incubation medium. Histamine was measured by a fluorimetric procedure as previously described (Boarato *et al.*, 1984). The release was expressed as a percentage of total cell histamine obtained by heating the mast cell suspension for 5 min at 100°C in 0.1 N HCl. The histamine release in the

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samples incubated without secretagogues did not exceed 3–5% of the total histamine in the various experiments.

Drugs

Apomorphine hydrochloride, tetradecanoylphorbolacetate (TPA), compound 48/80 and substance P were from Sigma Chem. Co. Melittin was from Serva. Freshly-made apomorphine solutions were maintained at 0°C in the dark and used without delay. Lysophosphatidylserine (Bigon *et al.*, 1979) and mouse β-nerve growth factor (Bruni *et al.*, 1982) were prepared as described.

Results

The secretagogues used in this study are shown in Figure 1. Confirming previous results, it was found that ATP and nerve growth factor plus lysophosphatidylserine elicited histamine release in the presence of extracellular calcium (Diamant & Kruger, 1967; Bruni *et al.*, 1982). Compound 48/80 showed equivalent activity either in the presence or in the absence of external calcium (Pearce, 1982) whereas the effects of TPA, melittin and substance P were optimal in a calcium-free medium (Battistella *et al.*, 1985; Fewtrell *et al.*, 1982; Pearce & Clements, 1982). As shown in Figure 2, apomorphine inhibited the histamine release induced by secretagogues requiring or

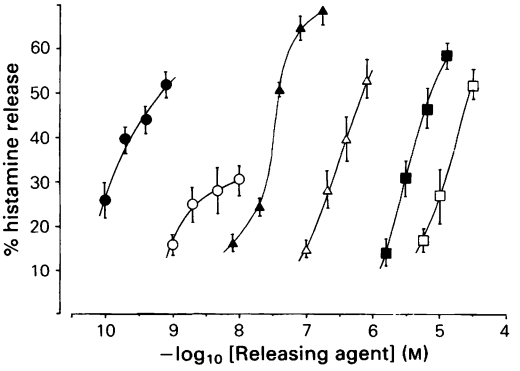

Figure 1 Log concentration-response curves for the histamine release induced by nerve growth factor plus 1 μM lysophosphatidylserine (●), tetradecanoylphorbolacetate (○), compound 48/80 (▲), melittin (△), substance P (■) or ATP (□). The points are the means from 4 experiments and the vertical lines show s.e.mean. Compound 48/80, nerve growth factor plus lysophosphatidylserine and ATP were assayed in the presence of 1 mM calcium. An average molecular weight of 1200 was assumed for compound 48/80 (Read & Lonney, 1972).

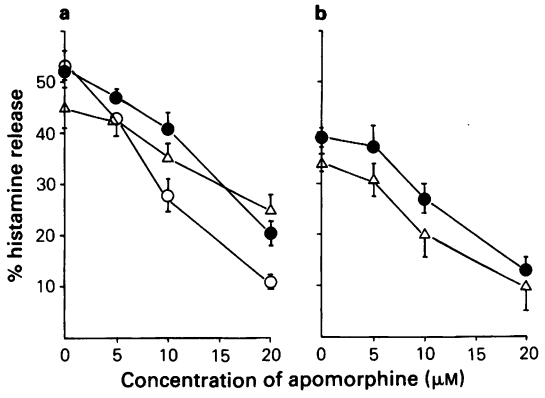

Figure 2 Inhibition of histamine release by apomorphine: mast cells were preincubated for 10 min at 37°C with the indicated concentrations of apomorphine before the addition of secretagogues. (a) ATP 30 μM (●), nerve growth factor 0.4 nM plus lysophosphatidylserine 0.5 μM (○), compound 48/80 0.04 μM (△) in a medium containing 1 mM calcium. (b) Substance P 3.4 μM (●), melittin 0.4 μM (△) in a calcium-free medium. Means from 3–6 experiments; vertical lines show s.e.mean.

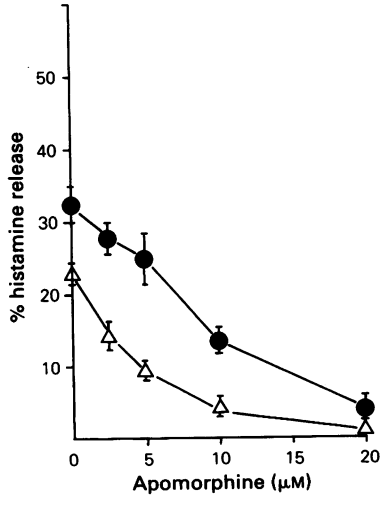

Figure 3 Inhibition of tetradecanoylphorbolacetate-induced histamine release. Mast cells were preincubated for 10 min with apomorphine before the addition of tetradecanoylphorbolacetate 2 nM in a calcium-free medium (●), or a medium containing 0.2 mM calcium (△). Means of 3 experiments; vertical lines show s.e.mean.

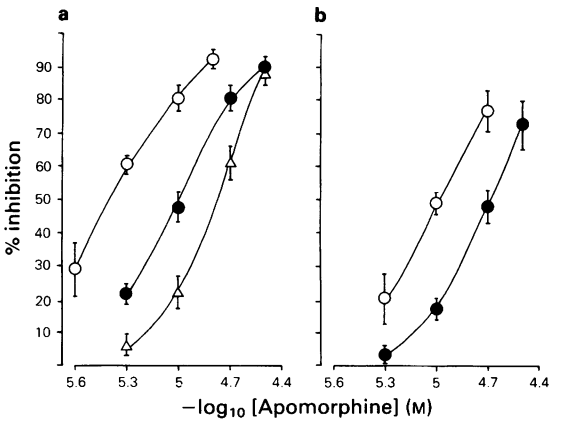

Figure 4 Inhibition of histamine release by apomorphine at increasing agonist concentrations: (a) 1 mM calcium 0.4 nM nerve growth factor and 0.05 μM (○), 0.5 μM (●) and 1 μM (△) lysophosphatidylserine. The % histamine release in the absence of apomorphine was 28.7 ± 3.9, 57.9 ± 4.2 and 66.4 ± 4.4, respectively. (b) Calcium-free medium with 2 μM (○) or 10 μM (●) substance P. The % histamine release in the absence of apomorphine was 24.5 ± 3 and 53.4 ± 4.0. Mean of 5 experiments; vertical lines show s.e.mean.

not requiring extracellular calcium. Provided the concentration of melittin was maintained at a level inducing approximately 30% of histamine release, apomorphine also prevented the non-specific effect of this compound. Similar observations on melittin were made with sodium cromoglycate as an inhibitor (Pearce & Clements, 1982).

Tested in a calcium-free medium, apomorphine was also effective on TPA-induced histamine release (Figure 3). The inhibition by apomorphine increased upon the addition of calcium, which, however, decreased the activity of this secretagogue. Calculation of the apomorphine median inhibitory concentrations (IC_{50}) under the conditions of Figures 2 and 3 revealed that the TPA-induced release was the most sensitive (IC_{50} of 8 μM). This value decreased to 4 μM in the presence of 0.2 mM calcium. Nerve growth factor plus lysophosphatidylserine (IC_{50} of 11 μM), melittin (13 μM), substance P (16 μM) and ATP (17 μM) showed approximately the same sensitivity. Apomorphine was rather less effective on the histamine release induced by compound 48/80 in spite of the addition of calcium to the incubation medium (IC_{50} of 24 μM). Subsequent experiments showed that the high effectiveness of apomorphine on the TPA-induced histamine release is explained by the limited potency of this secretagogue. As shown in Figure 4a, when the concentration of

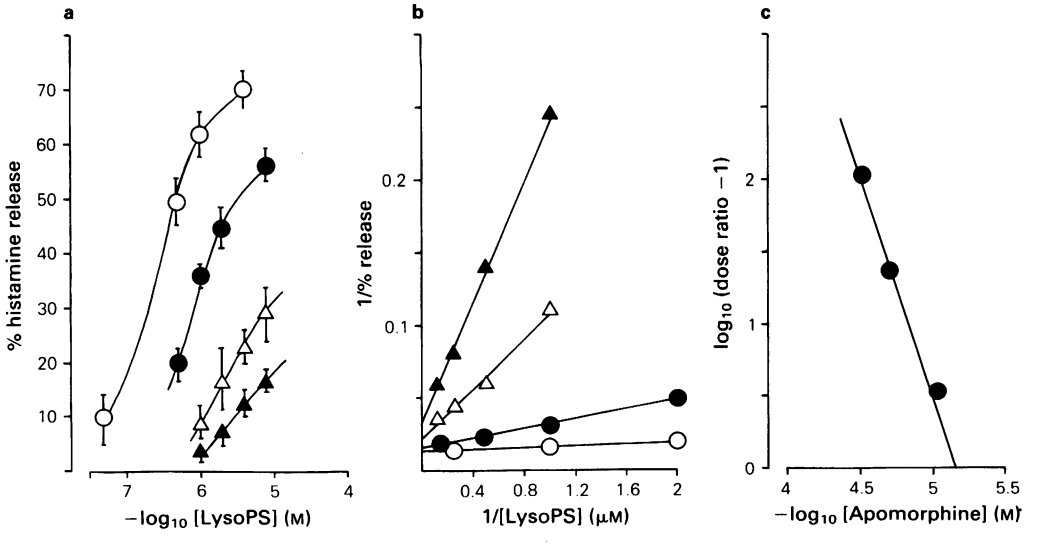

Figure 5 Concentration-response curves for lysophosphatidylserine (LysoPS) in the presence of 0.4 nM nerve growth factor. (a) Response without apomorphine (○), with apomorphine at 10 μM (●), 20 μM (△) and 30 μM (▲). Mean of 4 experiments; vertical lines show s.e.mean. (b) The same data plotted as reciprocals of lysophosphatidylserine concentration (abscissa scale) and of % histamine release (ordinate scale). Lines were obtained by linear regression analysis. Values of intercept ± 95% confidence interval: 0.013 ± 0.000 (○); 0.014 ± 0.008 (●); 0.020 ± 0.017 (△); 0.031 ± 0.004 (▲). (c) Schild plot for the inhibition by apomorphine at 20% histamine release. Slope ± s.e., 3.12 ± 0.25; pA_2, 5.15; pA_{10}, 4.85. Line calculated by regression.

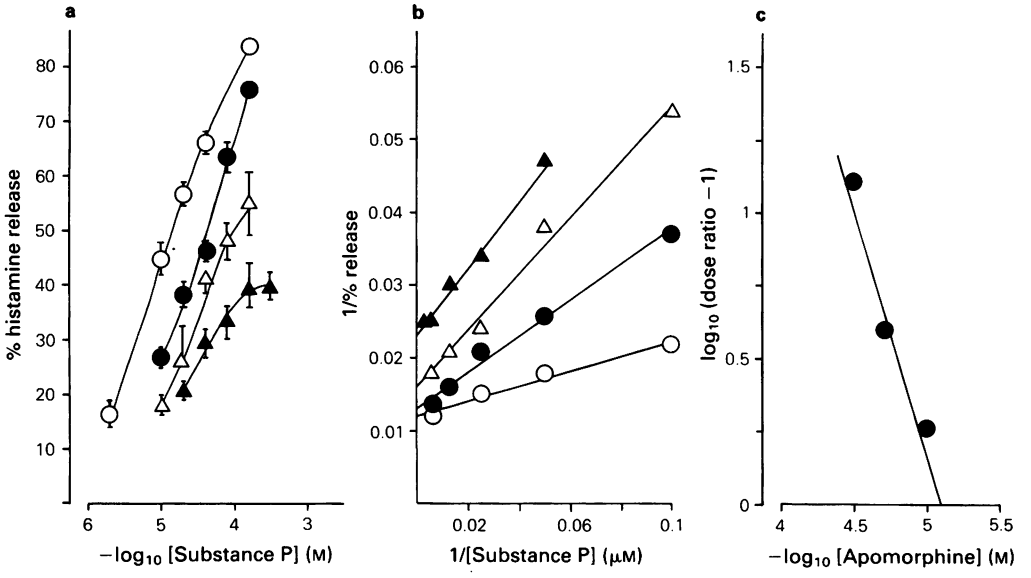

Figure 6 Concentration-response curves for substances P. (a) Response without apomorphine (○), with apomorphine at 10 μM (●), 20 μM (△), 30 μM (▲). Mean of 4 experiments; vertical lines show s.e.mean. (b) The same data plotted as reciprocals of substance P concentration (abscissa scale) and of % histamine release (ordinate scale). Lines calculated by regression. Values of intercepts ± 95% confidence interval: 0.012 ± 0.004 (○); 0.013 ± 0.003 (●); 0.016 ± 0.003 (△); 0.023 ± 0.003 (▲). (c) Schild plot for the inhibition by apomorphine at 35% histamine release. Slope ± s.e. 1.71 ± 0.48; pA_2, 5.1; pA_{10}, 4.5. Line calculated by regression.

lysophosphatidylserine added to nerve growth factor was decreased from 1.0 μM to 0.5 and 0.05 μM, the apomorphine IC_{50} changed from 17 μM to 11 and 4 μM, respectively. Under these conditions the percentage histamine release varied from 66.4 ± 4.4 to 57.9 ± 4.2 and 28.7 ± 3.9. This latter value was comparable to the effect of TPA. With substance P as an agonist the IC_{50} changed from 20 to 10 μM when the percentage histamine release decreased from 53.4 ± 4 to 24.5 ± 3 (Figure 4b).

Since the data of Figure 4 indicated an antagonism between apomorphine and secretagogues, this aspect was investigated in greater detail, using two different approaches. In the former, concentration-response curves for different secretagogues were obtained in the presence of 10 μM, 20 μM and 30 μM concentrations of apomorphine. In the latter, mast cells were pretreated with the drug (23 nmol per 10^6 cells), washed and challenged with increasing concentrations of histamine releasers. This procedure eliminated the possibility of chemical interaction between apomorphine and secretagogues in the incubation medium. Preliminary tests demonstrated that the apomorphine-induced inhibition was retained in the washed mast cells without changes in the total content of histamine. As shown in Figures 5 and 6, the concentration-response curves for either lysophosphatidylserine (in

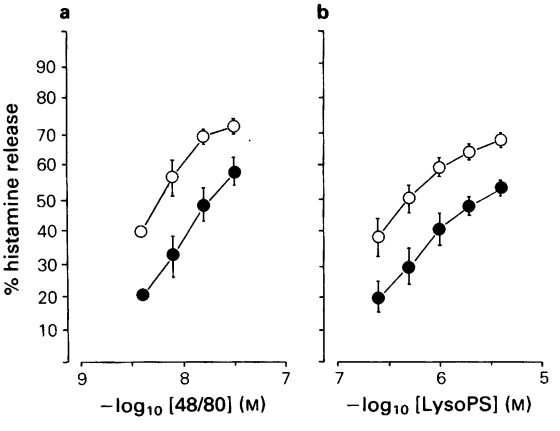

Figure 7 Agonist concentration-response curve in mast cells pretreated with apomorphine. A suspension of purified mast cells (3×10^6 cells ml^{-1}) was incubated for 10 min at 37°C with 70 μM apomorphine in a medium containing 1 mM calcium. After centrifugation and washing, the mast cells were stimulated with increasing concentrations of compound 48/80 (a) or lysophosphatidylserine (LysoPS) (in the presence of 0.4 nM nerve growth factor) (b). Untreated cells (○), apomorphine-treated cells (●). Mean of 3 experiments; vertical lines show s.e.mean.

the presence of nerve growth factor) or substance P were shifted to the right by apomorphine. With substance P the maximal response was decreased at 30 μM apomorphine, whereas with lysophosphatidylserine firm conclusions could not be drawn on this point because of the damaging effect of this compound on mast cells at concentrations higher than 10^{-5} M. In Figures 5 and 6 the data are also shown in the form of double reciprocal and Schild plots (Arunlakshana & Schild, 1959). In the double reciprocal plots, apomorphine increased both slopes and intercepts, the difference in the intercepts becoming significant at 30 μM concentrations of the drug. The Schild plots gave straight lines of slope higher than unity. According to the pA_2 values (determined from the abscissa intercept), apomorphine was an equipotent inhibitor of lysophosphatidylserine and substance P responses. Figure 7 shows that the apomorphine pretreated cells were less responsive to compound 48/80 and lysophosphatidylserine (in the presence of nerve growth factor) in spite of the washing procedure. Again, the histamine release was partly restored as the agonist concentration was raised.

Discussion

In this study, secretagogues were selected in order to investigate distinct pathways of mast cell activation. Nerve growth factor represents the group of agents requiring extracellular calcium and serine phospholipids for optimal activity (Bruni *et al.*, 1982; Boarato *et al.*, 1984). On the other hand, compound 48/80 and substance P elicit histamine secretion through the mobilization of intracellular calcium stores (Pearce, 1982; Fewtrell *et al.*, 1982). ATP is a secretagogue increasing the permeability of the mast cell membrane to ions without inducing loss of intracellular proteins (Cockroft & Gomperts, 1979a). TPA, the known activator of protein kinase C (Nishizuka, 1984), has the unique property of eliciting histamine release without increasing the free cytosol calcium concentration (White *et al.*, 1984). To this list of specific mast cell activators, melittin has been added as an example of a secretagogue producing a gross alteration of the mast cell membrane and loss of lactate dehydrogenase (Pearce & Clements, 1982). The data show that apormorphine inhibits the histamine release with all these compounds, melittin included. The conclusion can be therefore drawn that the drug does not affect the early process of histamine secretion such as surface receptor activation or calcium mobilization. Rather, a common regulatory step, controlling the terminal sequence of degranulation is likely to be affected. The inhibition of TPA-induced histamine release suggests that the apomorphine-sensitive step is closely associated with protein kinase C activity.

A further result of this study is the observed antagonism between apomorphine and mast cell activators. As a result, the potency of the drug declines at the highest values of histamine release. As seen in Figures 5, 6 and 7, apomorphine shifts the concentration-response curves for three different secretagogues to the right. Double reciprocal plots of percentage histamine release against the concentration of agonist show that the drug increases both slopes and intercepts. In addition, plotting the agonist dose-ratios against the concentration of apomorphine (Schild plot), straight lines of slope higher than unity were obtained, yielding a pA_2-pA_{10} difference of 0.6 with substance P and 0.3 with lysophosphatidylserine. These observations suggest that the apomorphine-induced inhibition is non-competitive, a conclusion in line with the finding that the drug produces similar effects on agonists expected to activate different receptors on the mast cell surface. Since the inhibition is retained in mast cells pretreated with apomorphine and washed, it is likely that the partial restoration of histamine secretion obtained at increasing concentrations of secretagogues is not due to apomorphine displacement but rather to the activation of alternative sequences linking the external stimulus to the secretory response. This conclusion is supported by the existing synergism among different pathways of mast cell activation. Indeed, it has been shown that the compounds promoting calcium influx or mobilization of intracellular calcium may converge in the activation of the phosphatidylinositol cycle (Kennerly *et al.*, 1979; Cockroft & Gomperts, 1979b). This, in turn, provides the diacylglycerol needed to activate the protein kinase C (Nishizuka, 1984). It is relevant in this context that the secretory response of mast cells to TPA does not exceed 35% of total histamine showing that the direct activation of protein kinase C mobilizes only a fraction of the secretory mechanism. Under these conditions the addition of lysophosphatidylserine that is inactive in rat mast cells when used alone, may increase the histamine release (Battistella *et al.*, 1985). The same effect is observed with a subliminal concentration of a calcium ionophore (Katakami *et al.*, 1984). Reduced efficacy at high concentration of secretagogues has been also observed with other mast cell inhibitors such as quercetin and sodium cromoglycate (Pearce, 1982; Pearce & Clements, 1982).

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