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CYCLIC AMP INDEPENDENT INHIBITION BY PAPAVERINE OF HISTAMINE RELEASE INDUCED BY COMPOUND 48/80*

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Abstract—Compound 48/80-induced histamine release from isolated rat peritoneal mast cells was inhibited in a dose-dependent manner by papaverine (IC_{50} approx 20 μM). This effect of papaverine was not influenced by PGE_1 (14–140 μM), even though PGE_1 markedly increased mast cell cAMP levels. Papaverine (0.5 mM) completely inhibited histamine release without causing any change in cAMP levels. Theophylline (0.1 and 0.5 mM) potentiated histamine release induced by submaximal concentrations of compound 48/80, while cAMP levels were increased. IBM X was as potent as papaverine in causing inhibition of mast cell phosphodiesterase. IBM X (0.14–0.7 mM) had no effect on histamine release but caused a 6–20 fold increase in mast cell cyclic AMP. Papaverine inhibition of histamine release was gradual at the onset and was parallelled by a depletion of mast cell ATP content. The inhibition of 48/80-induced histamine release and depletion of mast cell ATP levels was reversed by glucose. It is concluded that papaverine induced inhibition of 48/80-induced histamine release is independent of cAMP, is unrelated to phosphodiesterase inhibition but is dependent upon inhibition of energy production.

Anaphylactic histamine release from isolated rat peritoneal mast cells, human lung tissue and human leucocytes is depressed by agents that elevate cAMP levels [1–3]. The evidence suggesting a modulatory role of cAMP in anaphylactic histamine release was recently summarized [4].

There are similarities as well as dissimilarities between anaphylactic histamine release and release caused by the synthetic polyamine compound 48/80 [5, 6]. Loeffler *et al.* [7] found that dibuturyl cAMP, prostaglandin E_1 (PGE_1) and certain phosphodiesterase inhibitors decreased compound 48/80-induced histamine release from isolated rat mast cells. The authors suggested that both anaphylactic and compound 48/80-mediated histamine release are modulated by cAMP. On the other hand, Johnson and coworkers [8], who compared mast cell histamine release with cAMP levels failed to detect any clear relationship between these two parameters.

Papaverine is a well-known smooth muscle relaxant and a potent inhibitor of cyclic nucleotide phosphodiesterase [cf. 9]. These facts have been taken as evidence both that cAMP is involved in smooth muscle relaxation and that papaverine acts pharmacologically by elevating intracellular cAMP [9]. However, papaverine inhibits not only phosphodiesterase but also numerous other cellular enzymes and processes, including mitochondrial respiration [10].

We report that papaverine inhibits histamine release from isolated mast cells. This inhibition of histamine release seems to be independent of phosphodiesterase inhibition or changes of the cAMP levels. The inhibition was related to lowered mast cell ATP levels and thus probably to inhibition of energy production

MATERIALS AND METHODS

Male Sprague–Dawley rats (Anticimex strain) weighing 350 to 450 g were used. Mast cells from peritoneal and thoracic cavities were isolated by the Ficoll density gradient technique of Uvnäs and Thon [11]. The cells were 85 to 96% pure mast cells as judged by cell counting of stained or unstained cell populations.

After isolation, the cells were incubated at 37° in a medium containing 145 mM NaCl, 2.7 mM KCl and 10% (v/v) Sørensen phosphate buffer pH 7.0. Histamine release was induced by compound 48/80 (AB Leo, Helsingborg, Sweden) either by adding a small amount of a concentrated 48/80 solution to a dilute cell suspension or by adding an aliquot (1.5 μl) of a concentrated mast cell suspension to 2 ml medium containing compound 48/80. The final concentration of 48/80 was 0.5 μg/ml except where otherwise indicated. The histamine release was completed 1 to 5 min after addition of compound 48/80 [11]; the reaction was then stopped by chilling the tubes in an ice-water

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Abbreviations used: PGE_1, prostaglandin E_1; cAMP, adenosine-3′,5′-cyclic monophosphate; dibuturyl cAMP, N^6-2′-*O*-dibuturyl-adenosine-3′,5′-cyclic monophosphate; IBM X, 1-methyl-3-isobutylxanthine; ATP, adenosine 5′-triphosphate.

bath. Thereafter cells were separated from the medium by centrifugation (400 *g* for 5 min) at 4°. The cell cake was lysed in distilled water and resuspended by a Vortex-mixer. Histamine concentration in the medium and in lysed cells was measured as described previously [12]. Histamine release is expressed as per cent of total histamine content and was found to range between 1 and 7.2 per cent under basal conditions and between 42.5 and 81.6 per cent when stimulated by 0.5 μg/ml 48/80. The total amount of histamine in mast cells prepared by this procedure ranged between 12 and 24 μg per 10^6 cells. The influence of drugs was studied by preincubating the cells for 10–15 min before initiating the histamine release by compound 48/80.

For cAMP determinations 0.5–1 × 10^6 cells were deproteinized with 0.3 N perchloric acid (PCA). After centrifugation the clear supernatant was added to a 0.7 × 1.5 cm column of Dowex 50 W × 4 (200–400 mesh) in hydrogen form. The cyclic AMP was eluted with water. The cAMP containing fraction was lyophilized and resuspended in assay buffer [13]. cAMP was measured according to Brown *et al.* [13] using bovine adrenal binding protein. By means of internal standards corrections could be made for incomplete recovery (80–90%).

Cyclic nucleotide phosphodiesterase was measured according to Rutten *et al.* [14]. Concentrated mast cell suspensions (6–10 × 10^6 cells/ml) were incubated with drugs as indicated in the text. After 15 min of incubation the cell suspension was sonicated. An aliquot (45 μl) of the crude homogenate was incubated in a total of 250 μl 50 mM Tris–HCl, 10 mM NaAc, 2 mM $MgAc_2$ pH 7.5 containing 0.75–200 μM cAMP and 0.25 μCi ^{3}H-cAMP for 10–45 min at 30°, during which time the assay was linear. The reaction was stopped by placing the reaction tube in a boiling water bath for 2.5 min. 25 μl 0.2 mg/ml 5′-nucleotidase was added and the incubation continued for an additional 20 min. Following reheating and centrifugation a 200 μl aliquot of the supernatant together with carrier adenosine was added to a 0.7 × 1.5 cm column of Dowex-1-X8. Nucleosides were eluted with 10 ml 0.1 M $NaHCO_3$. Five ml eluant was mixed with 10 ml Instagel® (Packard) and the radioactivity counted in a Packard Tri-Carb liquid scintillation spectrometer.

Mast cell ATP content was determined as described by Peterson [15]. Three to five ATP determinations were made on each sample, standard or blank. Human serum albumin was included during the isolation and incubation procedures in order to increase mast cell stability. This made protein determinations meaningless and results are consequently expressed per million cells (or billion cells in the case of ATP).

Materials. Ficoll was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden), human serum albumin from AB Kabi (Stockholm, Sweden) and bovine serum albumin (Fraction V) from Sigma Co. (St. Louis, Miss., USA). Dowex 50 W-X 4 and Dowex-1-X8 were obtained from Kebo (Stockholm, Sweden) and were extensively washed with HCl and NaOH before use. 5′-Nucleotidase (Crotalus adamanteus toxin) was obtained from Sigma (St. Louis, Miss., USA), and ^{3}H-cAMP (27.5 μCi/m-mole) from the Radiochemical Centre (Amersham, England). Nucleotides and nucleosides were obtained from Boehringer (Mannheim, BRD). Papaverine hydrochloride (ACO, Sweden) was dissolved in distilled water, theophylline (ACO, Sweden) and 1-methyl-3-isobutylxanthine (Aldrich) in a minimal amount of acid. Dipyridamol (Boehringer-Ingelheim, BRD) and prostaglandin E_1 (Upjohn, Kalamazow, Mich., USA) were dissolved in ethanol. (The ethanol concentration in the incubation with mast cells never exceeded 10 mg/ml, a concentration known not to affect mast cell histamine [6]).

RESULTS

Effects on histamine release. A dose-dependent inhibition of histamine release was observed when dilute mast cell suspensions were preincubated with papaverine for 5 min before the addition of compound 48/80 (Fig. 1). The histamine release induced by 1 μg/ml 48/80 (a dose giving close to maximal histamine release as seen in Fig. 1) was inhibited by approximately 50 per cent by 10–20 μM papaverine and almost completely by 100 μM papaverine. A double reciprocal plot of the data in Fig. 1 suggests a mixed type inhibition.

PGE_1 had a very slight inhibitory effect on compound 48/80-induced histamine release (12.7 ± 1.7% inhibition at 0.14 mM). The inhibitory effect of PGE_1 was not significantly affected by prolonging the time of preincubation to 30 min, even though the histamine release caused by 48/80 alone was decreased. The inhibitory effect of papaverine on histamine release was not significantly changed by PGE_1 (0.01–0.14 mM). Theophylline (0.12 mM) did not inhibit histamine release or alter the effectiveness of PGE_1 as an inhibitor. In another series of experiments concentrated suspensions of mast cells were preincubated with different phosphodiesterase inhibitors: theophylline, IBMX, papaverine or dipyridamol. After preincubation an aliquot was transferred to fresh medium containing 48/80. The results are shown in Fig. 2. Papaverine and the high concentration of dipyridamol

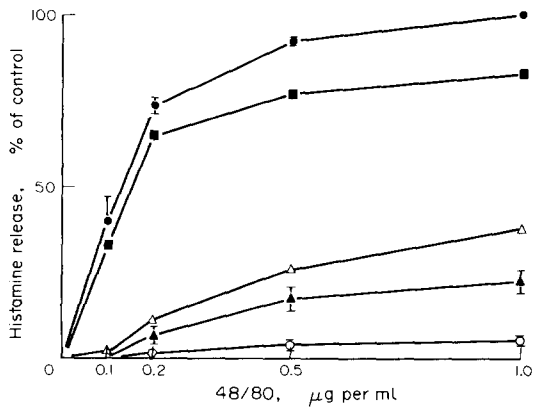

Fig. 1. Inhibition of compound 48/80 induced histamine release by papaverine. Results are expressed as histamine release in per cent of control release induced by 1 μg/ml 48/80 (70.4 ± 4%). ●, No papaverine (3 expts); ■, 0.01 mM papaverine (1 expt); △, 0.02 mM papaverine (1 expt); ▲, 0.033 mM papaverine (3 expts); ○, 0.1 mM papaverine (2 expts). Preincubation for 5 min before addition of 48/80.

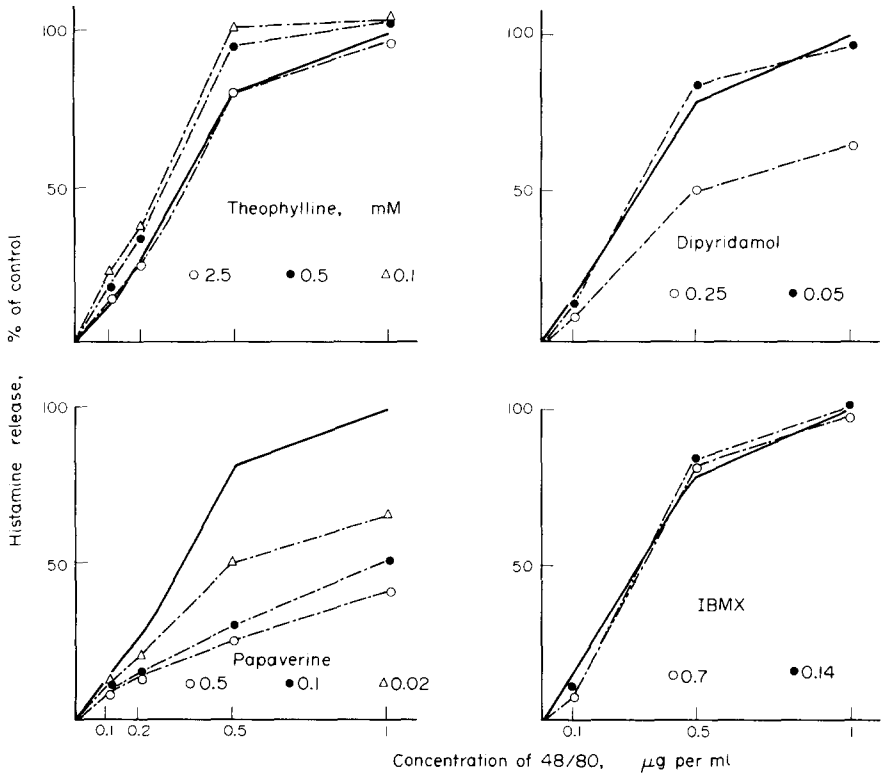

Fig. 2. The effect of some phosphodiesterase inhibitors on 48/80-induced histamine release. Concentrated cells were incubated with the drug for 15 min at the indicated concentrations. Results expressed as per cent of control release induced by 1 μg/ml 48/80 (46.7 ± 3.3%). Means of 3–6 determinations. Theophylline, △ 0.1 mM, ● 0.5 mM, ○ 2.5 mM; dipyridamol, ● 0.05 mM, ○ 0.25 mM; papaverine, △ 0.02 mM, ● 0.1 mM, ○ 0.5 mM; IBMX (3-isobutyl-1-methylxanthine), ● 0.14 mM, ○ 0.7 mM.

(0.25 mM) were inhibitory, but neither of the methylxanthines showed any inhibitory effect. In fact, the lower concentrations of theophylline enhanced the histamine release induced by suboptimal concentrations of 48/80. It can be calculated from the results shown in Fig. 2 that 0.1 mM theophylline increased release by 77% at 0.1 μg/ml 48/80, by 48% at 0.2 μg/ml 48/80, by 24% at 0.5 μg/ml 48/80, but only by 4% at 1 μg/ml 48/80.

Effects on cyclic AMP and phosphodiesterase. The cyclic AMP content of untreated mast cells was 0.80 ± 0.24 pmole/10^6 cells (n = 24). Papaverine (0.1 mM) caused an increase of 0.54 ± 0.26 pmole/10^6 cells (n = 20) over basal values. PGE_1 alone at a concentration of 0.14 mM caused a four-fold increase in the cyclic AMP content (to 3.49 ± 0.28 pmole/10^6 cells), and the addition of papaverine (0.1 mM) led to a further doubling (to 7.73 ± 2.27 pmole/10^6cells).

Table 1 shows the results of two experiments on the effect of four phosphodiesterase inhibitors on histamine release and on cyclic AMP content of mast cells. It can be seen that the two methylxanthines (theophylline and IBMX) had little or no effect on histamine release but caused a dose-dependent increase in cyclic AMP. In particular IBMX (0.70 mM) caused a 20-fold increase in cyclic AMP but no significant inhibition of histamine release. Papaverine, on the other hand, caused a marked inhibition of histamine release at 0.02 to 0.5 mM concentration, with a very minor effect on cyclic AMP. It is of interest that cyclic AMP elevations were more pronounced at low than at high concentrations of papaverine. These results demonstrate that histamine release caused by 48/80 can proceed normally even though cyclic AMP levels are elevated. They also show that papaverine inhibits histamine release independently of changes in cyclic AMP.

A kinetic analysis of mast cell phosphodiesterase activity revealed the presence of two forms, one with a K_m of about 1.5 μM and one with a K_m about 100 μM, in agreement with earlier results [8, 16]. The activity was found to be 0.08 and 1.8 nmole/min/10^6 cells at 1.5 μM and 100 μM cAMP, respectively, i.e. values similar to those reported by the other authors. The effects of increasing concentrations of the drugs are presented in Fig. 3. The IC_{50} for the low K_m enzyme (assayed at 1.5 μM cAMP) was 9 μM for IBMX, 14 μM for papaverine, 45 μM for dipyridamol and 1 mM for theophylline. The values are similar to those reported by Johnson *et al.* [8], who added the drugs directly to the assay medium.

Effect on ATP levels. The degree of inhibition caused by papaverine was far less when concentrated cell suspensions were preincubated with the drug and an aliquot transferred to fresh medium containing 48/80 (Fig. 2) than when dilute cell suspensions were used and the release reaction was initiated by a small aliquot of 48/80 added to the preincubation tube (Fig. 1). The difference does not appear to be due to a difference in cell concentration (experiments not shown). On the other hand, as shown in Fig. 4, the inhibitory effect of papaverine was very rapidly

Table 1. Relationship between compound 48/80-induced histamine release and cAMP content in mast cells treated with different phosphodiesterase inhibitors

Drug	Concentration (mM)	Histamine release (% of total)	cAMP content (pmoles/10^6 cells)
None	—	46.1 ± 1.2	0.58 ± 0.27
Theophylline	0.1	43.7 ± 0.3	0.38 ± 0.12
	0.5	40.9 ± 0.2	0.95 ± 0.15
	2.5	41.2 ± 0.3	3.16 ± 0.25
Papaverine	0.02	29.2 ± 1.0	1.67 ± 0.32
	0.1	27.6 ± 1.1	1.44 ± 0.52
	0.5	23.9 ± 1.4	0.61 ± 0.32
Dipyridamol	0.05	41.1 ± 1.4	2.91 ± 0.25
	0.25	26.9 ± 1.6	14.72 ± 1.92
Isobutylamethyl-xanthine	0.14	44.7 ± 0.3	7.30 ± 1.42
	0.70	44.9 ± 1.5	12.19 ± 2.40

Rat peritoneal mast cells (6.5–7 × 10^6 cells/ml) were incubated with or without drug for 15 min. 1.5 μl of the cell suspension was added to 2 ml buffer containing 1 μg/ml 48/80 (or no drug). After 3 min the reaction was stopped by cooling the tubes in ice water and histamine release was estimated as described in Methods. The remainder of the concentrated cell suspension was deproteinized by the addition of an equal amount of cold 0.6 N PCA, and used for the determination of cAMP. Mean ± S.D. from triplicate determinations in two experiments.

reversed when the cells were transferred to a fresh medium. Moreover, the inhibitory effect of papaverine on histamine increased progressively with time of preincubation of the drug. Such a time course is to be expected if papaverine acts by depleting the cell of an essential cofactor in the release reaction. Fig. 5 shows that papaverine caused a progressive depletion of mast cell ATP levels, which were reduced to less than half the original concentration within 2 min and almost to zero after 10 min. The results summarized in Fig. 6 show that this effect of papaverine can be counteracted dose-dependently by glucose. The results in Fig. 7 further demonstrate that papaverine and papaverine plus glucose produce essentially parallel effects on histamine release and on mast cell ATP levels.

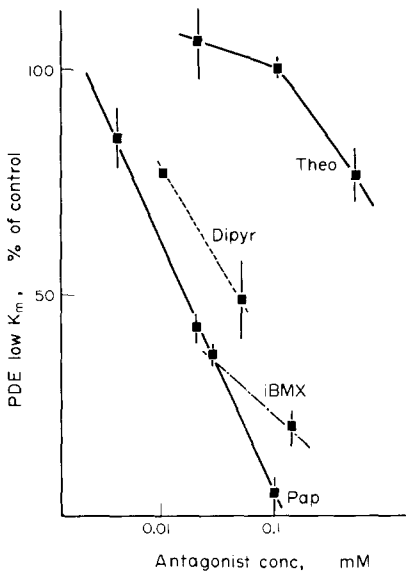

Fig. 3. Inhibition of low K_m phosphodiesterase by different drugs. Per cent of control PDE activity. Mean ± S.E.M. of duplicate determination from 2 or 3 separate experiments.

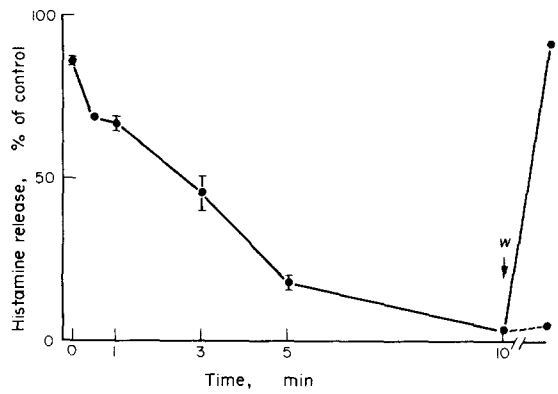

Fig. 4. Time course of inhibition by papaverine. Dilute cell suspensions were incubated with 0.1 mM papaverine for the time indicated along the abscissa before the addition of 48/80 (0.5 μg/ml). At W the cells were centrifuged and resuspended in fresh buffer either without (solid line) or with papaverine (broken line). The cells were then incubated for an additional minute before the addition of 48/80.

DISCUSSION

Several studies have examined the possible role of cyclic AMP on the histamine release process in mast cells. In some of these, cyclic AMP levels have been measured. In the present study, using a protein binding assay, we found, the cAMP level in untreated mast cells averaged 0.80 pmole/10^6 cells. This value is close to that reported by Johnson and coworkers (0.68 pmole/10^6 cells) [8], using a protein-kinase activation assay and by Gripenberg *et al.* (1.33 pmole/10^6 cells) [17], using radioimmunoassay. However, it is lower than the value reported by Kaliner and Austen (4.7 pmole/10^6 cells) [1] using a procedure similar to

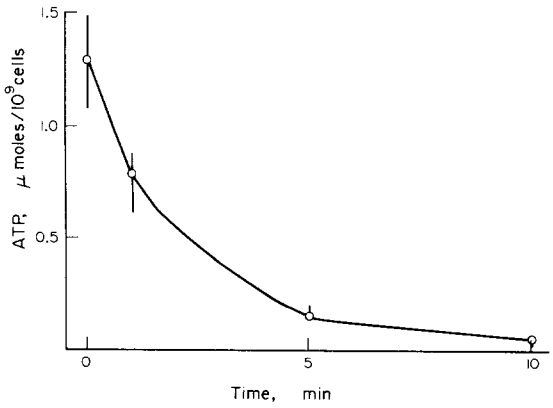

Fig. 5. Depletion of mast cell ATP by papaverine (0.1 mM). Mast cells were incubated for varying periods of time with the drug. ATP content was measured as described under Methods. Mean (± range) of three separate experiments.

ours and 20 times lower than the values reported by Sullivan *et al.* (16 pmole/10^6 cells) [18] using a radioimmunoassay. We have no explanation for the discrepancies between reported values for cAMP in untreated mast cells.

There appears to be general agreement that PGE_1, theophylline and papaverine elevate mast cell cAMP levels (Refs. 1,8,18 and present results), even though the absolute magnitude of the changes and the concentrations of the drugs necessary to induce them seem to vary somewhat between laboratories. In our hands, a high concentration of PGE_1 (0.14 mM) caused a very small, but statistically significant inhibition of 40/80 induced histamine release.

It is not possible to potentiate the slight inhibitory effect of PGE_1 by theophylline (Ref. 7 and present data). Theophylline in a concentration that caused a 4-fold increase in cAMP concentration did not inhibit 40/80-induced histamine release. In fact the effect of submaximal concentrations of 48/80 was considerably enhanced. We did not find any inhibition of 48/80-induced histamine release by IBMX, a potent phosphodiesterase inhibitor, even at concentrations

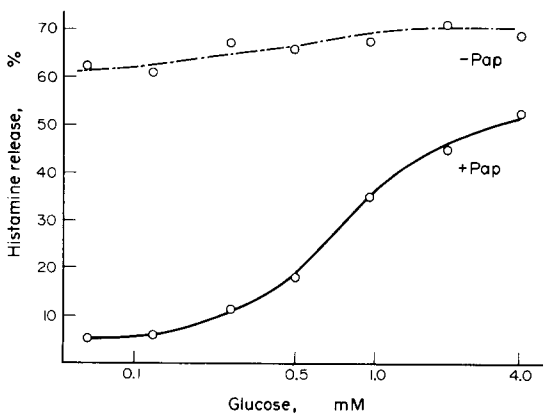

Fig. 6. Reversal of papaverine-induced inhibition of histamine release by addition of glucose. The mast cells were incubated at the indicated glucose concentrations with or without papaverine (0.1 mM) for 10 min before the addition of compound 48/80 (0.5 μg/ml).

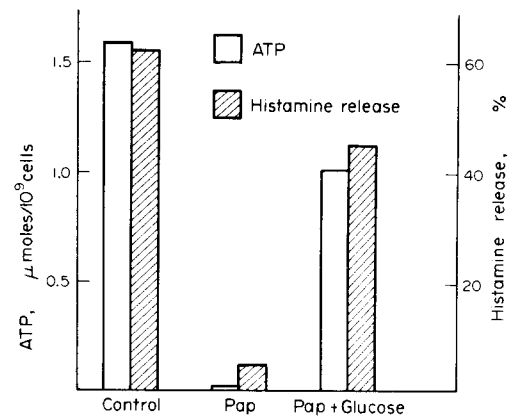

Fig. 7. Relationship between ATP content and histamine release. Mast cells were incubated in the absence (control), or in the presence of 0.1 mM papaverine (pap) or with 0.1 mM papaverine plus 2 mM glucose (pap + glucose) for 10 min before ATP determination or the addition of 48/80 (0.5 μg/ml).

causing a 20-fold increase in cyclic AMP. Johnson *et al.* [8], studying a number of drugs, failed to detect any clear relationship between changes in cAMP and changes in 48/80-induced histamine release. Our own results confirm this conclusion.

When mast cell suspensions were incubated with papaverine for 5 min or longer, 48/80-induced histamine release was dose-dependently inhibited with an IC_{50} of approximately 20 μM, which is close to the IC_{50} for phosphodiesterase inhibition by papaverine. Loeffler *et al.* [7] found a correlation between the concentration of theophylline, reserpine, DEAE-reserpine and perphenazine necessary to inhibit phosphodiesterase and 48/80-induced histamine release. They suggested that these drugs acted by preventing the breakdown of intracellular cAMP. The results presented in the present paper do not support this contention, however. Firstly, there is, as noted above, no clear evidence that increasing intracellular cAMP leads to an inhibition of 48/80-induced histamine release. A maximally effective concentration of papaverine (0.5 mM) had no effect on cAMP, while lower concentrations (0.02 and 0.1 mM) causing less inhibition of release did produce significant elevations of mast cell cAMP. Secondly, our data do not suggest a clear relationship between inhibition of histamine release and of phosphodiesterase. In particular, IBMX caused a marked inhibition of phosphodiesterase without affecting histamine release.

The inhibition of histamine release by papaverine was gradual in onset but rapidly reversed upon washing and was overcome by glucose. Johnson *et al.* [9], who used 5.6 mM glucose in the medium, found no significant inhibition by papaverine. These results suggest that papaverine inhibits energy metabolism in mast cells. There is considerable evidence that histamine release induced by compound 48/80 is an energy-dependent process and that inhibition of mast cell energy production can inhibit histamine release (for references see [15]). Browning *et al.* [19] recently reported a concentration dependent inhibition of respiration by papaverine (IC_{50} 5 μM) in C-6 astrocytoma cells as well as a time-dependent depletion of

creatine phosphate and later of ATP. We also found a time-dependent depletion of mast cell ATP content. The time-course for depletion of ATP and inhibition of 48/80-induced histamine release was quite similar (Figs. 4 and 5). Furthermore, the reversal of papaverine inhibition of histamine release by glucose is paralleled by a restoration of ATP levels (Fig. 7).

In conclusion, we have found a concentration dependent inhibition of 48/80 induced histamine release by papaverine. This is probably not due to an inhibition of phosphodiesterase leading to increased intracellular cAMP levels, since there was no correlation between cAMP content of mast cells and the histamine release (Ref. 8 and present results) or between inhibition of histamine release and inhibition of mast cell phosphodiesterase. In contrast, our data suggest that papaverine acts via depletion of mast cell ATP, leading to an inhibition of energy requiring processes, into which category 48/80-induced histamine release falls.

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