Effects of Mepacrine and p-Bromophenacyl Bromide on Anti-IgE and Phospholipase A2-Induced Histamine Release from Human Basophils*

J.B.C. TOLL and R.G.G. ANDERSSON
Department of Pharmacology, University of Linköping, S-581 85 Linköping

Abstract
Exposure of human basophils to purified phospholipase A2 caused a release of histamine, the process could be divided in one Ca²⁺-independent and one Ca²⁺-dependent stage. Low concentrations of mepacrine and p-bromophenacyl bromide (BPB) inhibited both phospholipase A2- and anti-IgE-induced histamine release. Mepacrine was more potent than BPB when the two-stage-method was used. The inhibition of mepacrine was most effective when the drug was added in the second Ca²⁺-dependent stage. The effect of mepacrine in the whole reaction of the anti-IgE-induced histamine release was biphasic and mepacrine was less effective than in the inhibition of the separated stages. The effect of BPB on the whole reaction was rather similar to mepacrine, although it was not biphasic. The results also suggest that inhibition of histamine release due to inhibition of phospholipase A2 might be of therapeutic value as the system can be inhibited at very low drug concentrations.

Histamine can be released from mast cells and basophils by both immunological and non-immunological stimuli. The histamine release, induced by specific antigens or by anti-IgE, has been extensively investigated. It has become widely accepted that histamine secretion from mast cells and basophils is dependent on the presence of extracellular Ca²⁺ ions [1, 2]. The role of intra- and extracellular calcium in the histamine release has also been discussed by Pearce et al. [3]. Influx of Ca²⁺ from the extracellular environment into the cell is a sufficient stimulus to initiate histamine secretion; e.g. the ionophore A 23187 induces histamine release by stimulation of transmembrane movement of calcium into the cells [2, 4]. The antigen-induced, IgE-mediated release of histamine from basophilic cells can be divided into two steps: 1. an antigen-dependent, Ca²⁺-independent activation step and 2. an antigen-independent, Ca²⁺-dependent release step [5].

Recently, it has been suggested that the histamine release of mast cells is correlated with a phospholipid methylation and an activation of phospholipase A2 [6-8]. Phospholipase A2 is a Ca²⁺-dependent enzyme which has been shown to release histamine from mast cells in the presence of extracellular Ca²⁺ [6]. However, it has been suggested that this histamine release is induced by a cytotoxic action or by contaminants in the preparation of phospholipase A2 [9-12]. Phospholipase A2 can be blocked by mepacrine and BPB [6, 8, 13, 14]. Results showing different effects of mepacrine and BPB on the histamine secretion from rat mast cells have been presented by Nemeth and Douglas [15].

In this work we have investigated the histamine release induced by phospholipase A2 in human basophils. We have also compared the effects of mepacrine and BPB on anti-IgE and phospholipase A2-induced histamine release. In some experiments we have divided the releasing process into two steps [5] to get more information about the inhibition in presence and absence of extracellular Ca²⁺ ions.

Material and methods
Venous blood for human volunteers was subjected to
sedimentation with 6% dextran containing 6 mM EDTA and the leukocytes were isolated as previously described [16]. The leukocytes were washed twice in Tris-A buffer solution (2 mM Tris, 120 mM NaCl, 5 mM KCl, 0.3 mg/ml human albumin; pH = 7.4). After the last wash the cells were resuspended in Tris-A or Tris-ACM (Tris-A + 0.6 mM CaCl$_2$ and 1 mM MgCl$_2$). In most experiments, the procedure for two-stage histamine release [5] was used with minor modifications.

**First stage inhibition**

Leukocytes, suspended in Tris-A buffer, were pre-incubated for 10 min at 37°C whereafter mepacrine or BPB was added. Mepacrine and BPB were obtained from Sigma Chemical Co., St. Louis, Mo., USA. BPB was dissolved in dimethylsulfoxide (DMSO) and diluted with Ca$^{2+}$-free Locke solution (150 mM NaCl, 5 mM KCl, 1 mM MgCl$_2$ and 3 mM Na$_2$HPO$_4$/NaH$_2$PO$_4$; pH = 7.1). After an incubation for 15 min anti-IgE or phospholipase A2 (porcine pancreas, Sigma), was added to the solution. The cells were incubated for 4 min when anti-IgE was used as releasing agent and for 15 min when phospholipase A2 was used. (This incubation will be referred to as stage 1). After the incubation the cells were immediately centrifuged and washed three times with Tris-A buffer before they were resuspended in Tris-A buffer. The leukocytes were then incubated for 10 min at 37°C, and 0.6 mM of Ca$^{2+}$ and 1 mM of Mg$^{2+}$ were added to start the releasing process. This incubation (referred to as stage 2) was allowed to go on for 40 min.

**Second stage inhibition**

Leukocytes, suspended in Tris-A buffer, were pre-incubated for 10 min at 37°C. Anti-IgE or phospholipase A2 was added and the cells were incubated as described above (stage 1). After the cells had been washed they were incubated for 10 min and then treated with mepacrine or BPB for 15 min. After the addition of Ca$^{2+}$ and Mg$^{2+}$ the cells were incubated as described above (stage 2).

**Determination of histamine secretion**

After the incubation, the cells were centrifuged and the histamine, released in the supernatant, was determined by a fluorometric technique [17, 18]. Corrections were made for spontaneous histamine release and have been deducted from the values presented. Complete histamine values were obtained by treating the cells with perchloric acid.

**Results**

Human basophils, incubated with phospholipase A2 in a Ca-free medium, released histamine when incubated with Ca$^{2+}$ ions in stage 2. The histamine release was dependent on the concentration of phospholipase A2 and the time of incubation. Figure 1a shows the dose-response curve for the effect of phospholipase A2. The time of incubation was 15 min in stage 1 and 30 min in stage 2 in these experiments. In some experiments, the time of incubation in stage 1 varied between 5 and 30 min. Optimal results were obtained after 15 min of incubation, while shorter or longer times of incubation gave less histamine release. The time course of the second incubation is shown in Figure 1b. The cells were activated by the addition of optimal doses of phospholipase A2 for 15 min in stage 1. Ca$^{2+}$-requirement for anti-IgE- and phospholipase A2-induced histamine release were also tested. The role of extracellular Ca$^{2+}$ ions in the second stage 2.