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Inhibition of anaphylactic histamine release by complexes of lidocaine with zinc

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A protective effect of zinc ions in experimental allergy was shown earlier by us [1] and others [2]. *In vitro* experimental evidence indicates that zinc ions stabilize the membrane of rat peritoneal mast cells and significantly inhibit disruption of these cells by compound 48/80, lecithinase A or antigen–antibody interactions, to prevent histamine release [3, 4].

It has been reported that some local anaesthetics, among them lidocaine also exert an inhibitory effect on histamine release from mast cells [5]. Since lidocaine has ligand properties and can complex with metal ions, two complexes of zinc with lidocaine have been recently prepared [6] in order to combine the inhibitory action of both constituents. We showed that these complexes were more potent than lidocaine and zinc alone in inhibiting histamine release induced by compound 48/80 [7] and the ionophoreous antibiotics A 23187 and X 537A [8].

Here we present evidence that they are also potent inhibitors of anaphylactic histamine release.

Materials and methods. Female F1 hybrids of August and Wistar rats weighing 200–250 g were used for immunization. The antigen used was a dissociated hemocyanin according to White and Holm [9]. Aluminium hydroxide gel was used as an adjuvant/(60 mg $Al(OH)_3$ per ml).

Rats were injected subcutaneously into the back and the foot pads with 500 μg hemocyanin in 1 ml adjuvant [10]. 21 to 30 days after sensitization, mast cells from the abdominal and thoracic cavities were harvested and isolated in a Ficoll density gradient [11]. After isolation of the mast cells they were washed 3 times by centrifugation at 200 g for 5 min using a medium of following composition: 144mM-NaCl, 2.7mM-KCl, 1mM-$CaCl_2$, 2mM-glucose, 1 mg/ml of bovine serum albumin, buffered with Sörensen phosphate buffer 6.7 mM to pH 6.9–7.0. The same medium was used for incubation.

Cells were incubated for 5 min in 2 ml aliquots at 37° in the presence of tested compounds and then antigen (20 μl) was introduced and incubation continued for 10 min.

Following incubation the cells were centrifuged at 350 g for 10 min. The supernatants were decanted into new tubes and 2 ml 0.08 N HCl was added to the cell sediments to lyse the cells.

Histamine was determined fluorometrically according to Shore *et al.* [12] omitting the extraction procedure [13].

Histamine release was calculated as a percentage of the total histamine content of each cell sample. In all experiments triplicate samples were carried out and the mean values were used for calculations. The spontaneous release of histamine amounted to 2.9 ± 0.8 per cent; this has been deducted from all values presented.

The complexes of zinc with lidocaine tested were: a coordination complex in which lidocaine (Lid) is directly bound to metal by an oxygen atom ($ZnLidCl_2$) and an ionic complex in which metal is coordinated with chloride ions and lidocaine occupies an outer coordination sphere ($ZnCl_2HLid_2$, see ref. 6).

Giant keyhole limpet hemocyanin was obtained from Schwartz/Mann (Orangeburg, N.Y.)

Results and discussion. Antigen-induced histamine release in controls amounted to 23.9 per cent (Fig. 1). In the presence of tested agents at concentrations of 10^{-5} M histamine release was significantly inhibited by the ionic complex (14.7% release) whereas the coordination complex only slightly depressed the reaction (18.5% release); at 5×10^{-5} M both complexes markedly inhibited release (54% and 46% for ionic and coordination complex, respectively).

At 10^{-2} M ionic complex completely inhibited antigen-induced release and coordination complex gave 55 percent inhibition.

These results indicate that the ionic complex of lidocaine with zinc is more potent than the coordination complex in inhibition antigen induced histamine release from rat mast cells. From the best fitting pair of parallel regression lines the potency ratio is about 3. A similar pattern of the action of zinc

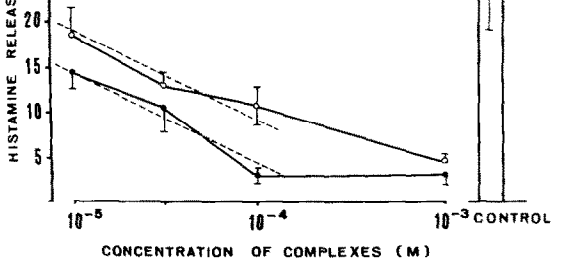

Fig. 1. The effect of zinc complexes with lidocaine on antigen-induced histamine release. O———O; coordination complex; ●———●: ionic complex. Each point represents mean ± S.E. of 4 experiments. Regression lines are marked with dotted line.

complexes with lidocaine has been noted using compound 48/80 as a histamine releaser [8].

However, when histamine was released by the ionophores, the inhibitory action of the coordination complex was more pronounced [8] possibly indicating differences in the mechanism of histamine release by antigen or 48/80 on one hand and by ionophores on the other.

Investigated compounds were introduced into a physiological medium (pH 7.0) containing an excess of Cl^- ions. In these conditions Zn(II) ions in both complexes act presumably as $(ZnCl_4)^{2-}$ anions and lidocaine is a protonated form; hence a stronger inhibitory effect than that of lidocaine or zinc alone [7] is obtained.

Considering the inhibitory effect of complexes against broad spectrum of mast cell secretagogues (antigen, compound 48/80, A 23187, X 537A) their interaction with releasing agents seems to be unlikely. Alternatively they may act on mast cells to protect them against various releasers by stabilizing cell membranes. This cromoglycate-type mechanism of action possibly could be helpful in treating allergic disorders.

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The effect of chlorpromazine and 6-hydroxydopamine on arachidonic acid metabolism *in vitro*

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The role of free radicals in prostaglandin (PG) biosynthesis has not been elucidated. It was described that free radicals are inhibitors of cyclo-oxygenase [1–4] since some compounds acting as free radical scavengers are stimulators of prostaglandin generation. On the other hand chlorpromazine, which is also a free radical scavenger [5] has been found to inhibit PG biosynthesis [6–9]. In order to elucidate the role of free radicals in PG biosynthesis we compared the influence of chlorpromazine on this synthesis with that of 6-hydroxydopamine, a known free radical generator [5]. All the methods used have been described previously [10]. The only difference was that unlabeled standards of PGE_2, $PGF_{2\alpha}$ and 6-keto

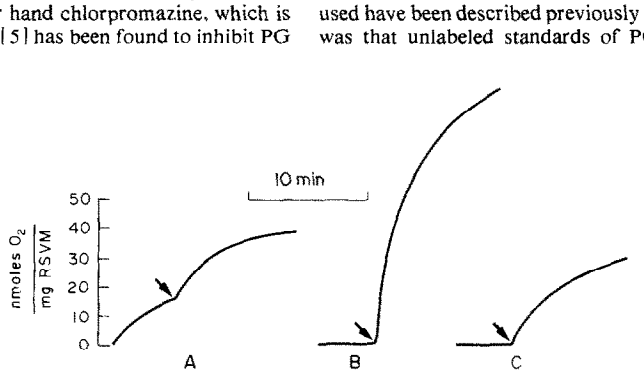

Fig. 1. Tracings of a typical experiment on the influence of chlorpromazine and 6-hydroxydopamine on oxygen consumption by ram seminal vesicle microsomes (RSVM). A. Control incubation mixture containing 1.5 mg microsomes/ml in 0.1M-Tris buffer pH 8.2 and sodium arachidonate (added as indicated by arrow) at the final concentration of 100 μM. B. The same as A + 1000 μM of chlorpromazine added at the beginning of the experiment. C. The same as A + 100 μM of 6-hydroxydopamine added at the beginning of the experiment.