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SHORT COMMUNICATIONS

The action of the complexes of lidocaine with zinc on histamine release from isolated rat mast cells

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Earlier investigations show that zinc ions exert an inhibitory effect on rat mesenteric mast cell disruption [1] and on histamine release from mast cells [2, 3] produced by compound 48/80. The protective effect of zinc ions on guinea pig experimental bronchial asthma [4] and on human air tract obstruction [5] also has been reported.

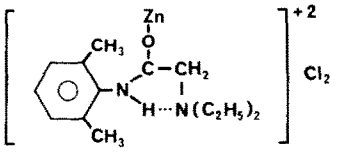
Hydrated cations Cu (II), Pb (II), La (III), and other metals also inhibit histamine release from mast cells [1, 2, 6]. Zinc ions are of low toxicity relative to other transition metals [7]; moreover their selective accumulation in mast cells has been observed [8, 9]. Hence further tests on zinc application in inflammatory processes, including allergies, seemed warranted.

It is interesting that zinc ions bound to the organic ligand 8-hydroxyquinoline, at 1:1 and 1:2 molar ratios, elicit a greater stabilizing effect on biomembranes than do free ions [10, 11]. Some membrane depolarization blocking agents, among them local anaesthetics [12] exert a similar inhibitory effect on histamine resease from mast cells. Some of these anaesthetics have ligand properties and can complex with metal ions. Possibly such complexes would be helpful in treating allergic disorders by inhibiting release of allergic mediators, inhibiting the vagal reflex, decreasing tonus of bronchial smooth muscle and reducing vascular permeability.

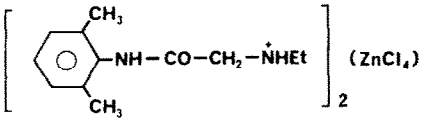
The aim of the present work was to test newly-prepared complexes of zinc with lidocaine (Lid = lidocaine): these contain a ligand directly bound to metal by an oxygen atom ($ZnLidCl_2$) or a form of cation complexed with $ZnCl_4^{2-}$ ($ZnCl_4/HLid/_2$), where zinc is coordinated with chloride ions and lidocaine is placed in outer coordination sphere. The proposed formulas for the salts examined are shown in Fig. 1.

MATERIALS AND METHODS

Mast cells were obtained from the peritoneal cavity of August rats, both sexes (200–300 g) by lavage. For each experiment cells from two to three rats were pooled, washed and resuspended in buffer medium (pH 6.8–6.9) containing NaCl 154 mM, KCl 2.7 mM, $CaCl_2$ 0.9 mM, glucose 10 mM, human serum albumin 1 mg/ml and 10% (v/v) of HEPES buffer.



$ZnLidCl_2$



$ZnCl_4(HLid)_2$

Fig. 1. Chemical formulas of zinc complexes with lidocaine.

Mast cells were isolated according to Johnson and Moran's method [13] by use of 37% bovine serum albumin (fraction V). Cells on the surface of the albumin layer were removed by section and the cells in albumin were washed three times in fresh medium. Finally they were pooled and divided into 10–20 aliquots for incubation. The cell samples were preincubated in a metabolic shaker at 37° for 5 min in the presence of tested agents. Then compound 48/80 was added (final concentration 0.5 μg/ml) and incubation continued for 10 min. The incubates were then cooled to 4° and cells separated by centrifugation at 250 *g* for 5 min. Supernatant samples were saved for histamine estimation. The cell sediment was treated with 0.3 ml of distilled water, cooled to the temperature of dry ice, thawed, the volume made up to original (3 ml) with fresh medium and the histamine content determined.

Histamine was determined by the biological method of Code [14], using the guinea pig's isolated atropinized ileum (0.3 μg atropine sulphate per ml Tyrode's solution). Mepyramine was used to identify histamine (0.4 μg mepyramine maleate per ml Tyrode's solution). The samples were diluted 15-fold before assay; under these conditions the presence of test agents did not influence the response of guinea pig's ileum.

Drugs. We are greatly indebted to Dr. Dziudziel and professor Łodzińska, Institute of Chemistry, Copernicus University, Toruń, Poland for the preparation of both complexes of zinc with lidocaine. Zinc chloride was purchased from BDH, lidocaine hydrochloride from SIMS, and compound 48/80 from Sigma.

RESULTS AND DISCUSSION

The effects of zinc-lidocaine complexes on compound 48/80-induced histamine release from rat mast cells are shown in Fig. 2.

The ionic complex $ZnCl_4$ $(HLid)_2$ was the strongest showing inhibition of 70% at 10^{-4} M, 90% at 5×10^{-4} M, and a significant effect at 10^{-5} M. The coordination complex $ZnLidCl_2$ was slightly weaker and showed no significant inhibition at 5×10^{-5} M; at 5×10^{-4} M about 90% inhibition was observed.

Free zinc ions gave still weaker inhibition, whereas lidocaine alone, except at high concentrations, was virtually without effect. The response to all four tested substances was dose-dependent. We have shown that both types of lidocaine-zinc complexes have a more distinct inhibitory effect on histamine release compared to free zinc ions and especially lidocaine alone. A similar effect was found with zinc complexed with another ligand, 8-hydroxyquinoline [10]. The data are not compatible with activity of complexes due to a synergistic effect of zinc plus lidocaine.

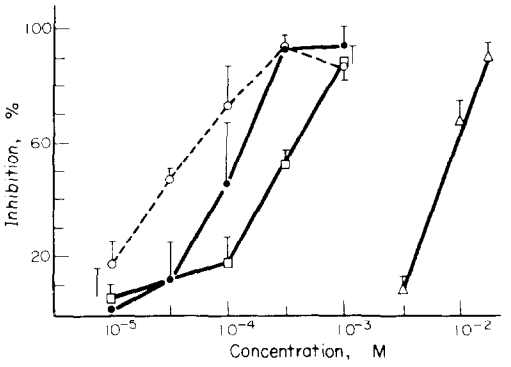


Fig. 2. The inhibitory effects of zinc, lidocaine and complexes between them on rat mast cell histamine release induced by compound 48/80 (0.5 μg/ml). Mean value (± S.D.) from 4–5 experiments are expressed as per cent inhibition. For each experiment 2–3 rats were used. O- -O zinc-lidocaine ionic complex ($ZnCl_4/HLid_2$); ●- -● zinc-lidocaine coordination complex ($ZnLidCl_2$); □- -□ zinc chloride; △ —△ lidocaine hydrochloride.

The potency of the complexes may arise from close binding to the cell membrane; a slow continuous release of zinc ions at this strategic position could favour membrane stability [11, 15]. A possible interaction of compound 48/80 with zinc in the complexes should also be considered. However, earlier experiments with zinc [10] in which inhibition of histamine release was higher when mast cells were preincubated with zinc before compound 48/80 addition than when compound 48/80 was preincubated with zinc before an addition of mast cells rather exclude the direct inactivation of compound 48/80 by zinc. Planned studies of the effect of zinc-lidocaine complexes on antigen-induced histamine release in anaphylaxis may help to clarify this field.

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REFERENCES

1. B. Högberg and B. Uvnäs, *Acta physiol. scand.* **48**, 133 (1960).
2. B. Diamant and B. Uvnäs, *Acta physiol. scand.* **53**, 315 (1961).
3. W. Kazimierczak and C. Maśliński, *Agents and Actions* **4**, 1 (1974).
4. W. Kazimierczak, *Acta physiol. pol.* **25**, 609 (1974).
5. S. Kubiak, S. Słomka, W. Kazimierczak and C. Maśliński, *Balneol. Pol.* (in press).
6. J. C. Foreman and J. L. Mongar, *Nature, New Biol.* **103**, 255 (1972).
7. M. Chvapil, *Life Sci.* **13**, 1041 (1973).
8. R. Keller and E. Sorkin, *Experientia* **26**, 30 (1970).
9. H. Fiedler, H. Hahn von Dorsche, P. Fehrman and R. Sulzmann, *Histochemie* **24**, 150 (1970).
10. W. Kazimierczak and C. Maśliński, *Agents and Actions* **4**, 320 (1974).
11. M. Chvapil, J. N. Ryan and C. F. Zukoski, *Proc. Soc. exp. Biol. Med.* **140**, 642 (1972).
12. W. Kazimierczak, M. Peret and C. Maśliński, *Biochem. Pharmac.* **25**, 1747 (1976).
13. A. R. Johnson and N. C. Moran, *Proc. Soc. exp. Biol. Med.* **123**, 886 (1966).
14. C. Code, F. F. C. McIntire and D. Glick, *Meth. Biochem. Analysis* **3**, 49 (1959).
15. A. Albert, *Selective Toxicity*, p. 338. Methuen, London (1968).

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Glycolytic metabolites and adenosine triphosphate in skeletal and cardiac muscle of rats after clofibrate feeding

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An acute muscular syndrome was reported in patients treated with ethylchlorophenoxyisobutyrate [1], including myalgia, cramps, weakness and stiffness. This seems to be an uncommon side effect of clofibrate therapy [2], possibly correlated to higher doses [1] and free serum levels of the drug [3]. Even mild muscular effort may accentuate these side effects [4]. Similar symptoms were seen in patients suffering from a heriditary insufficiency of muscle phosphorylase, which causes an inadequate glucose supply from glycogen during muscle contraction [5]. Clofibrate reduces liver glycogen levels in animals [6, 7] and modifies the content of glycolytic metabolites in rat liver [8, 9]. In addition myotonia was induced in rats by clofibrate [10]. This could suggest an interference of clofibrate with glucose metabolism of muscle.

Female rats, Wistar strain, were used throughout. They were kept on a diet with 0.25% (w/w) clofibrate. (The diet was a gift of the ICI Industrial Company, Macclesfield, England). The mean weight of the animals was 210 ± 15 g and 222 ± 25 g (±S.E.) after 3 or 6 weeks of clofibrate feeding. The values of the control groups were 207 ± 15 g and 238 ± 25 g respectively.

Muscle of the hind leg and the whole cardiac muscle were freeze-clamped. The detailed analytical procedure is described elsewhere [9]. The U-test of Wilcoxon, Man and Whitney was used for statistical analysis.

A decreased glycogen content of rat skeletal muscle was reported also by Miyazawa *et al.* [8]. Lower glycogen values are also found in the cardiac muscle after clofibrate exposure [17.7 ± 1.3 μmoles glycosyl-units g wet wt^{-1} (mean ± S.E.M.), $n = 10$ in the experimental group, 25.1 ± 1.9 μmoles, $n = 9$ in the control group). The decrease of 29.5 per cent was significant ($P < 0.05$). The weight of the hearts did not differ in control (0.654 ± 0.14 g, mean ± S.E.) and the clofibrate group (0.645 ± 0.07 g). The mechanism of glycogen decrease during clofibrate treatment in rats is not yet completely understood. Some data are available to support a reduced glycogen synthesis [11]. Incorporation of ^{14}C-labelled glucose into glycogen is reduced in liver slices of clofibrate treated rats [11] and dogs [7]. The enzyme glycogen synthetase (Glycogen-UDP glycosyl-transferase EC 2.4.1.11] is activated by insulin via the modification of the protein kinase [12]. Immunoreactive insulin was reported to be reduced