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THE ACTION OF LOCAL ANAESTHETICS ON HISTAMINE RELEASE

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Abstract—The effect of the local anaesthetics lidocaine, procaine and tetracaine on compound 48/80-induced histamine release from isolated rat mast cells has been investigated. They inhibited histamine release in a dose-dependent manner; at a concentration of 20 mM there was almost total inhibition of histamine release by lidocaine and about 75% inhibition by procaine. Tetracaine exerted a biphasic effect: at concentrations below 1 mM it inhibited, but at concentrations above 1 mM it potentiated histamine release. The inhibitory effect of lidocaine on compound 48/80-evoked histamine release was dependent upon the time of preincubation of mast cells with this anaesthetic and it persisted after washing the cells and resuspension in a lidocaine-free medium. An increase of calcium ions antagonized the inhibitory action of lidocaine. These results can be explained by (1) blockade of membrane receptors for calcium binding which leads to a decrease in intracellular calcium concentration and (2) increase of cellular cyclic AMP content which subsequently inhibits the releasing process.

Local anaesthetics inhibit membrane depolarization by reducing sodium and potassium conductances [1, 2]. This seems to follow displacement of calcium ions from binding sites in the membrane [1–4]. Treatment with local anaesthetics can also lead to physical stabilization of membranes [5] and to inhibition of several kinds of cell interactions such as platelet adhesiveness [6, 7], cell fusion [8] or leukocyte adherance to endothelium [9].

Secretory processes also require calcium to unite the stimulus with the actual secretion. This aspect is interesting in that local anaesthetics cause a dose-dependent inhibition of both catecholamine release produced by calcium [10] and glucose stimulated insulin secretion [11]. This indicates that local anaesthetics can block calcium movement in the adrenal medulla or in beta-cells in a manner which parallels their inhibition of monovalent cations in electrically excitable tissue.

Histamine release also appears to be a secretory process in which calcium couples the stimulus to the exocytosis of histamine-containing granules [12]. Therefore local anaesthetics may be considered as inhibitors of histamine release. In this paper we present some data which supports this hypothesis.

METHODS

Rats weighing 200–300 g were exsanguinated by decapitation and the abdominal cavity was opened. Mast cells were harvested and processed in a medium containing 154 mM NaCl, 2.7 mM KCl, 0.9 mM $CaCl_2$, 10 mM glucose, 1 mg/ml human serum albumin and buffered to pH 6.9–7.0 with 10% (v/v) Tris buffer. The mast cell medium was prepared just before use. The opened abdominal cavity was flooded with 5 ml of medium and the abdomen of the animal was gently massaged for 2 min. The medium containing suspended peritoneal cells was recovered by siliconized pipette and placed on the surface of 37% bovine serum albumin (fraction V) in plastic tubes according to Johnson and Moran's method for mast cell isolation [13]. Finally the isolated mast cells from 4 or 5 rats were pooled. There was about 10–15% contamination by other peritoneal cells.

The pooled mast cells were divided into ten to twenty samples each containing approximately 200,000–300,000 mast cells. The samples were preincubated in plastic tubes (10 min, 37° and continuously shaken) with different concentrations of local anaesthetics. If it was necessary, the pH of the samples was adjusted to the original with 1N NaOH. After subsequent incubation with compound 48/80 for 5 min the samples were centrifuged for 5 min at 4°, and 250 *g*. Histamine was determined both in the supernatant (directly) and in the cells after extraction by freezing and thawing. When tetracaine alone was used as a histamine liberator the cells were exposed for 15 min.

In the experiments with lidocaine and procaine histamine was assayed according to Code [14] using guinea pig isolated atropinized ileum (0.3 μg atropine sulfate per ml Tyrode's solution). Agents used in the experiments were added to all histamine standards in respective concentrations for controls.

When tetracaine was tested, histamine was determined fluorometrically using the Lorenz's column chromatographic separation method [15]. The histamine released was expressed as a per cent of the total histamine content in the mast cells. Under these conditions there was 1–6% spontaneous histamine release.

The arithmetic mean, standard deviation and Student's *t*-test were used in the calculations.

RESULTS

The effects of adding lidocaine to the mast cell incubation medium 10 min prior to the histamine liberator compound 48/80 are shown in Fig. 1. At a con-

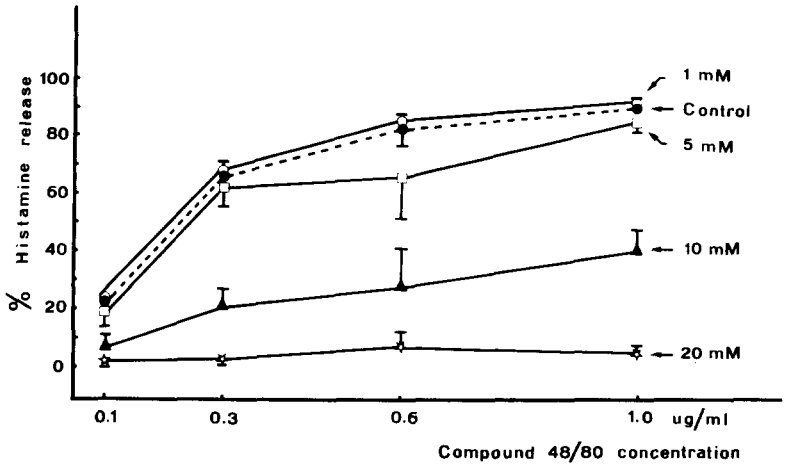

Fig. 1. Effect of lidocaine on compound 48/80-induced histamine release. Compound 48/80 was added directly to the mast cell incubation medium after 10 min preincubation with different concentrations of lidocaine. Samples without lidocaine are controls. Mean values (±S.D.) are expressed in per cent of total histamine in the cell samples. Each point is the mean of 3 double experiments from 4 rats.

centration of 1 mM lidocaine caused a slight potentiation of histamine release. However at 5 mM there was a slight but significant ($P < 0.01$) inhibition of histamine release at the compound 48/80 concentration of 0.6 μg/ml. With 10 mM lidocaine there was a significant decrease in histamine release at all compound 48/80 concentrations used. At the concentration of 20 mM there was a complete inhibition of histamine release and no correlation between amount of released histamine and 48/80 concentration.

To test whether the effect of lidocaine on histamine release depends on the Ca^{++} concentration, calcium ions were introduced as a calcium chloride to the mast cell incubation medium. The results presented in Fig. 2 show that Ca^{++} antagonize the effect of lidocaine. Lidocaine at a concentration of 10 mM markedly decreased the histamine release (about 81%) in Ca^{++} free medium. An increase in Ca^{++} concentration from 1 to 5 mM augmented histamine release from 25.9% to 56.5%. These data indicate that calcium ions play a significant role in lidocaine-modified histamine release and also that there is an antagonism between calcium ions and lidocaine.

Figure 3 shows that tetracaine and procaine also inhibit compound 48/80-induced release of histamine. The order of potency of inhibition of histamine release was: tetracaine > lidocaine > procaine, a good correlation with their local anaesthetic potency [16].

Tetracaine exerted biphasic effect (Fig. 4). At concentrations below 1 mM tetracaine inhibited histamine release but at higher concentrations it enhanced compound 48/80-evoked histamine release. Tetracaine alone also released histamine; it is probably a nonselective histamine liberator due to its high affinity with phospholipid constituents of the cell membrane [17]. This effect was concentration-dependent from 0.5 to 3 mM. At the concentration of 3 mM, tetracaine produced almost 100% of histamine release. It is interesting to note that tetracaine alone at the concentration of 1 mM acts as a histamine liberator and releases about 20% of histamine, but at the same concentration given together with compound 48/80 it acts as an inhibitor of histamine release.

DISCUSSION

Histamine in mast cells is stored in the cellular granules linked to a heparin–protein complex [18] and all of the available evidence favours the hypothesis that histamine release from mast cells occurs by expulsion of the granular material through the cell membrane. This process has been termed degranulation and appears to correspond to exocytosis in endocrine secretory systems [19]. Membrane stimulation appears to be associated with a change of permeability which can be measured by electrophysiological techniques. Indeed, depolarization of the rat mesentery mast cell follows the addition of 48/80 [20]. Although depolarization is not a causal phenomenon

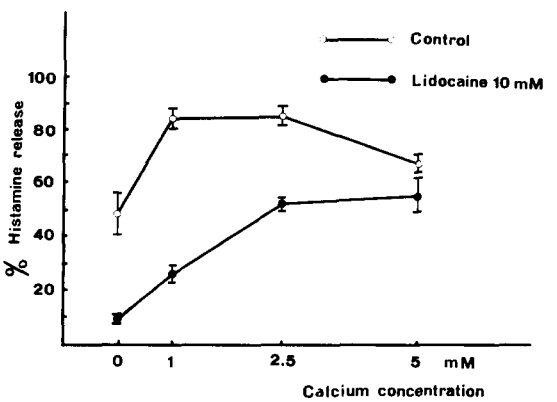

Fig. 2. Effect of calcium ions on inhibition of histamine release by lidocaine. Compound 48/80 (0.3 μg/ml) was used to histamine release. Mast cells were preincubated for 10 min with calcium chloride before compound 48/80 addition. The samples without lidocaine are controls. Mean values (±S.D.) are expressed as a per cent of total histamine in cell samples. Each point is the mean of 3 double experiments from 4 rats.

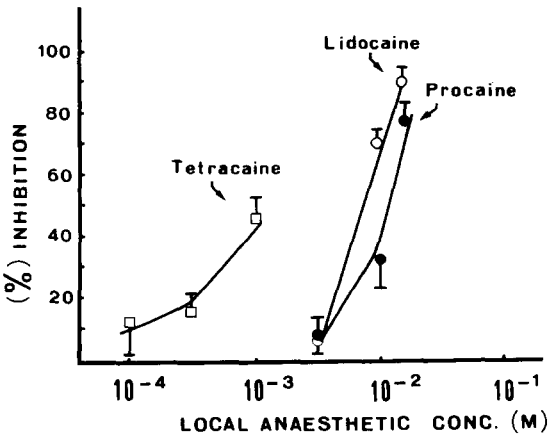

Fig. 3. The action of tetracaine, lidocaine and procaine on compound 48/80-induced histamine release. The mast cells were preincubated with the anaesthetics for 10 min before compound 48/80 addition (0.3 μg/ml). Mean values (±S.D.) are expressed in per cent of inhibition of histamine release. The amounts of histamine released in the absence of local anaesthetics were taken as a 100%. Each point is the mean of 4–5 double experiments in the case of tetracaine and 3 double experiments in the case of the other local anaesthetics. Usually 4 rats were used for each experiment.

in the mechanism of mast cell degranulation, but similar to other secretory systems is merely a secondary event, it, together with the observation that stimulation of the rat mast cell by 48/80 results in an increase in calcium and sodium uptake and enhancement of potassium release [21] indicates the similarities between secretory and excitatory processes. In relation to these findings it seems that the action of the local anaesthetics is based on the same mechanism both on nerve excitability and endocrine cell secretion.

The calcium dependence of the histamine releasing effect of 48/80 appears to vary with the tissue and species. Histamine release from isolated rat peritoneal mast cells by 48/80 seems to be less calcium dependent although the presence of calcium enhances the release (Fig. 2) [22]. Feinstein [23] and others [1, 2, 4, 24] reported that calcium ions have an affinity for phospholipids, especially phosphatidyl serine, and also that local anaesthetics strongly inhibit this linkage. On the other hand it is well known that phosphatidyl serine is an important constituent of the mast cell membrane which has been considered a membrane receptor for calcium ions [12]. It is quite possible that local anaesthetics exert their inhibitory action on histamine release by competitive inhibition of calcium ion binding to its membrane binding sites. Our experiment showing the protective effect of increasing concentration of exogenous calcium ions (Fig. 2) firmly supports this hypothesis.

The relatively high concentrations of local anaesthetics which effectively enhibited histamine release may suggest a problem of penetration. However these concentrations were lower than those commonly used in local anaesthesia. Furthermore the concentrations of local anaesthetics used in our experiments are comparable to the concentrations of the other antianaphylactic drugs such as disodium cromoglycate [25], theophylline [26] or nicotinamide [27].

It is known that a number of antihistamines exhibit good local anaesthetic properties. On the other hand as reported by Mota *et al.*[28] and quite recently by Lichtenstein and Gillespie, H_1 antihistamines especially phenothiazine derivatives [29] which possess strong local anaesthetic potency [30] not only block histamine receptors but also in low doses inhibit both antigen and compound 48/80 induced histamine release from mast cells and antigen induced histamine release from leukocytes. We believe that the mechanism of this action is similar to that presented by us on the action of other local anaesthetics.

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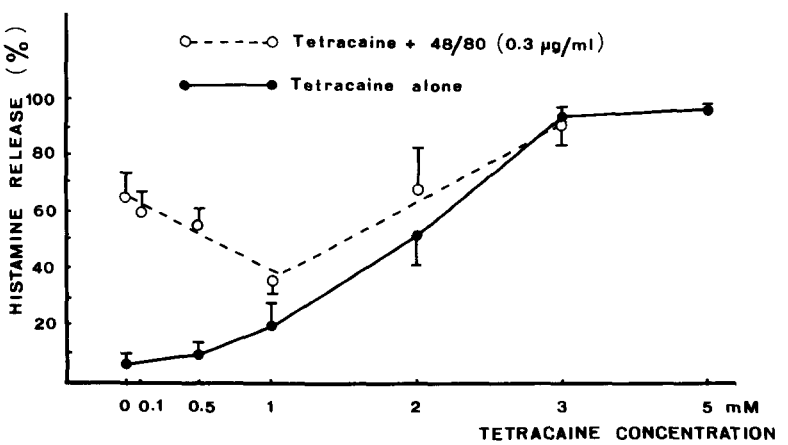

Fig. 4. A comparison of two opposite effects of tetracaine: as an inhibitor of histamine release and as a histamine releaser. In experiments in which compound 48/80 and tetracaine were tested together, the mast cells were preincubated for 10 min in the presence of tetracaine. When tetracaine was used alone the mast cells were exposed for 15 min. Mean values (±S.D.) are expressed in per cent of the total histamine content in the cell samples. Each point is the mean of 4–5 experiments from 4 rats.

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