Communications to the Editor

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IN SITU DETECTION OF HISTAMINE RELEASED FROM MAST CELLS BY USING A HISTAMINE-SENSITIVE MEMBRANE ELECTRODE

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We constructed a histamine-sensitive membrane electrode by using sodium tetrakis(*p*-fluorophenyl)borate as an ion-exchanger and *o*-nitrophenylphenylether as a membrane solvent and applied it in the detection of histamine released from mast cells. This is the first attempt to measure the histamine secretion process *in situ* without any separation of histamine from the assay medium.

KEYWORDS —— histamine; histamine-sensitive electrode; ion-selective electrode; membrane electrode; histamine release; mast cell; electrochemical analysis

Since Shore and co-workers reported the fluorometric assay of histamine in 1959,[1] this method is widely utilized to measure the histamine liberated from tissues or isolated mast cells.[2] Here, we report a new method for detecting histamine using a histamine-sensitive membrane electrode. This electrochemical analysis using a selective ion-sensitive electrode has the inherent advantage of being simple and easy, especially in continuous assays.[3-5] With this method it is possible to measure the histamine secretion process *in situ* without separating the histamine from the assay medium.

A histamine-sensitive membrane electrode was constructed by the use of a poly(vinyl chloride)-based membrane.[3-5] The components of the membrane are: 0.5 mg of sodium tetrakis(*p*-fluorophenyl)borate, 60 µl of *o*-nitrophenylphenylether, and 30 mg of poly(vinyl chloride). These materials were dissolved in 1 - 2 ml of tetrahydrofuran. The solution was poured into a flat Petri dish 30 mm in diameter, then the solvent was evaporated at room temperature. The resulting membrane was cut and stuck on a poly(vinyl chloride) tube (outer diameter: 4 mm, inner diameter: 3 mm) with tetrahydrofuran. The sensor membrane was conditioned overnight in a solution containing 1 mM histamine, 10 mM NaCl, and 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes)-NaOH (pH 7.4). The electrochemical cell arrangement was as follows: Ag, AgCl/ internal solution/ sensor membrane/ sample solution/ 1 M NH_4NO_3 (salt bridge)/ 0.01 M KCl/ Ag, AgCl. The internal solution was the same as the one used to condition the membrane. The electromotive force between the pair of Ag/AgCl electrodes was measured with an appropriate field effect

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transistor-operational amplifier (input resistance: $> 10^{12}\Omega$) and recorded.

Figure 1 shows the calibration curve of electric potential measured in physiologically balanced salt solution containing 154 mM NaCl, 2.7 mM KCl, 0.9 mM $CaCl_2$, 5.6 mM glucose, and 5 mM Hepes-NaOH (pH 7.4). The electrode responded to histamine even at concentrations below 0.1 mM. The selectivity coefficients $\log K_{HiX}^{Pot}$ of the electrode[6] were: Mg^{2+}, -3.8; Ca^{2+}, -3.8; Na^+, -2.4; H^+, -1.9; NH_4^+, -1.6; K^+, -1.2; $CH_3NH_3^+$, -0.5; serotonin, 0.3; $(CH_3)_4N^+$, 2.3. These were determined by the separate solution method[6] in 10 mM solutions of the respective chloride salts. A solution containing 10 mM histamine was adjusted to pH 7.4, in which the histamine existed as its monohydrochloride. It should be emphasized that the responses of the present electrode to many organic amines were stronger than that to histamine. The large interference from organic amines is characteristic of an ion-selective electrode prepared with a lipophilic ion-exchanger.[7,8] Nevertheless, we thought that the present electrode can be applied for the detection of histamine released from mast cells, since mast cells do not contain organic amines to any significant extent other than histamine.[9] An important problem was whether the present electrode can detect histamine released from mast cells. It was calculated that 15-30 μg of histamine can be obtained from 10^6 mast cells[10] which are usually collectable from the abdominal cavity of an individual rat. This amount seems to be sufficient, because

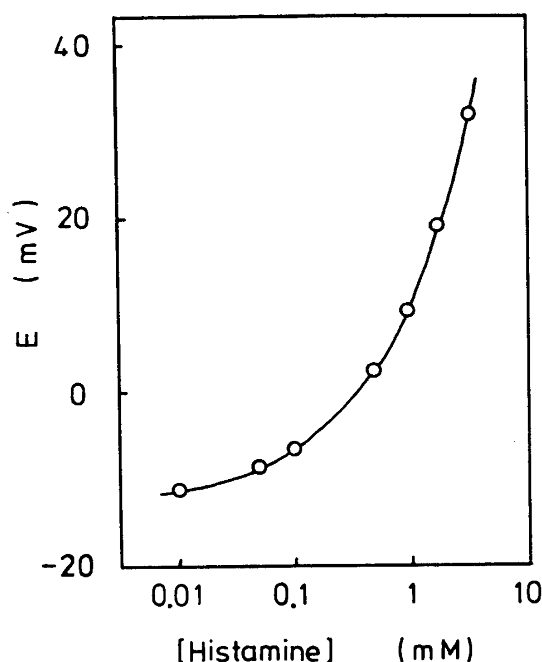

Fig. 1. Response of a Histamine-sensitive Electrode in Physiologically Balanced Salt Solution at 28°C

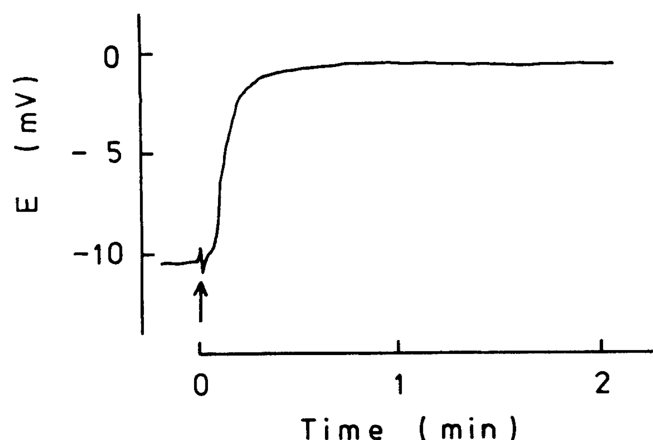

Fig. 2. Release of Histamine from Mast Cells Induced by Polylysine

Rat peritoneal mast cells (2×10^5 cells) were suspended in 200 μl of physiologically balanced salt solution containing 154 mM NaCl, 2.7 mM KCl, 0.9 mM $CaCl_2$, 5.6 mM glucose, and 5 mM Hepes-NaOH (pH 7.4) at 28°C. The arrow indicates the time when 2 μl of polylysine (final concentration: 50 μg/ml) was added.

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if we decrease the amount of assay solution down to 1 ml, the concentration of histamine will be around 0.2 mM. The present electrode system, including the reference electrode,[5] is compact, and it is easy to decrease the volume of an assay solution down to 0.2 ml. Figure 2 shows a typical tracing obtained during the measurement of histamine released from rat peritoneal mast cells. To induce histamine release, we added 50 μg/ml of polylysine (degree of polymerization, n = 20) to the cell suspension. It has been reported that polycations such as polylysine can induce the secretion of histamine from rat peritoneal mast cells.[11,12] After addition of polylysine, the rapid elevation of histamine concentration was clearly apparent, indicating that the present electrode system was adequate to measure the amount of histamine released from mast cells. Addition of polylysine alone into the medium did not induce any response.

So far, this is the first attempt to measure histamine secretion without any sampling or separation of histamine from an assay solution. This potentiometric method using an ion-selective electrode provides a new way to assay histamine, and offers a basic technique for kinetic studies of the time course of histamine secretion processes.

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