MEMBRANE POTENTIAL OF MAST CELLS AND HISTAMINE LIBERATION FROM THEM

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The results of measurement of the membrane potential of peritoneal mast cells and the effect of increasing the extracellular potassium ion concentration on the membrane potential and on the liberation of histamine from the mast cells are described. The membrane potential of mast cells in Krebs' fluid of normal salt composition was −11.6 ± 0.48 mV (from −7 to −25 mV), and when the extracellular potassium ion concentration was increased to 127 mM the membrane potential was −7 ± 0.38 mV (from −5 to −14 mV), indicating depolarization of the cell membrane under those conditions. The spontaneous liberation of histamine from the mast cells into normal and potassium-enriched Krebs' fluid was the same, and the ability to liberate histamine in response to addition of ATP (5 mM) was undisturbed in the potassium solution but was inhibited in the absence of potassium ions. It is concluded that depolarization of the mast cell membrane itself is not essential for triggering the mechanism of histamine liberation.

The mechanism of histamine liberation from the mast cells, judging by the morphological, biochemical, and some physiological parameters, is similar to the mechanism of secretion of mediators and hormones from effector cells [7, 8, 16]. In particular, the potentiating role of calcium ions in the secretion of biologically active substances is a universal feature of all these processes. However, the connection between the membrane potential of the cell and triggering the mechanism of liberation of mediators or hormones and with an increase in the conductance of the membrane for calcium ions as the initial component of this mechanism may vary from object to object. It is well known that depolarization of the cell membrane is the trigger mechanism for liberation of mediators from certain nerve endings. The secretion of catecholamines from the chromaffin cells of the adrenals is evidently triggered in the same way [6]. On the other hand, the induced secretion of certain hormones, especially steroids, from the cells of the adrenal cortex takes place without depolarization of the cell membrane and artificial depolarization does not induce such secretion [13]. No attempt has been made to study these mechanisms in mast cells, and what is more, no results of measurement of the resting membrane potential of these cells have previously been published.

This paper describes the results of direct measurement of the resting membrane potential of mast cells by means of intracellular microelectrodes and attempts to liberate histamine from them by artificially induced depolarization of the cell membrane.

EXPERIMENTAL METHOD

A suspension of peritoneal cells was obtained from male albino rats weighing 150-200 g after intraperitoneal injection of 10 ml Krebs' solution [1] with the addition of 1 mg/ml of human serum albumin as described previously [3]. The method of recording the membrane potential of the mast cells under the

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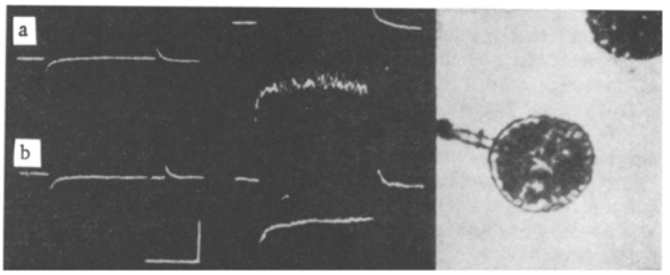

Fig. 1. Membrane potential of mast cells in Krebs' fluid of normal salt composition (a) and with increased concentration (127 mM) of potassium ions (b). On left: artifacts recorded by electrode in extracellular position on making and breaking electric current triggering microelectrode plunger; right – records obtained during introduction of microelectrode into and its withdrawal from mast cell. Downward deflection of beam corresponds to electronegativity. Vertical line – 10 mV, horizontal line – 0.5 sec. Photomicrograph on right shows mast cell at moment of penetration by microelectrode located above the cell, 600×.

control of an inverted microscope by means of glass microelectrodes with a resistance of about 100 MΩ was described in a special communication [2].

In each experiment a suspension of cells obtained from four rats was used to determine histamine. It was divided into eight equal portions, four of which were used for the subsequent determination of histamine (first row of tubes), while the other four (duplicates) were used for the morphological control (second row of tubes), in which the mast cells were stained with a 0.3% alcoholic solution of neutral red [3]. The cells were centrifuged for 5 min at 350 g. The supernatant was poured off, and 5 ml of Krebs' solution of normal salt composition was added to the first and second tubes of both rows, 5 ml of Krebs' fluid with an increase (to 127 mM) concentration of potassium ions was added to the third and fourth tubes (the NaCl was replaced by KCl in an equimolar concentration). The cells were then incubated at 37°C for 5 min, ATP was added to a final concentration of 5 mM to the second and fourth tubes of both rows, and all the portions of cells were incubated at 37°C for 10 min. The reaction was then stopped by placing the tubes in ice-cold water. The cells were centrifuged at 350 g for 5 min at 1-2°C. The supernatant, acidified with 0.1 volume N HCl, was used for determination of the histamine concentration. Histamine was determined in the residues of the first row after extraction by resuspending the cells in 5 ml distilled water, acidified with 0.1 volume N HCl, and boiling for 3 min. The residues of the second row of tubes were used as the morphological control. A similar scheme was used in experiments in which the effect of ATP (5 mM) in a calcium-free solution was tested. To prepare the solution, no calcium ions were added to the Krebs' fluid, and any traces of calcium present were bound by the addition of 1 mM EDTA; the pH of all the solutions was 7.3.

Histamine was determined spectrofluorometrically [14] in Pytskii's modification [4]. The corresponding buffer solutions with the addition of ATP were used as the control. The quantity of histamine liberated from the cells was expressed as a percentage of its total content in the portion.

EXPERIMENTAL RESULTS

Puncture of the mast cell by the microelectrode was accompanied by the stepwise appearance of a negative potential which disappeared when the electrode was moved in the other direction and withdrawn from the cell. A photomicrograph of a mast cell at the time of its puncture by the microelectrode located above the cell is shown in Fig. 1a. The mean value of the membrane potential of mast cells of a peritoneal suspension of rats in Krebs' solution of normal salt composition was -11.6 ± 0.48 mV (M ± m; from −7 to −25 mV; number of cells tested 84). A membrane potential above 15 mV was recorded in this group of tests in 20 cells; its value was -18 ± 0.71 mV.

With an increase in the extracellular potassium ion concentration to 127 mM, depolarization of the cell membrane was observed. The character of the potential recorded in the depolarizing potassium solution is shown in Fig. 1b. The mean value of the membrane potential recorded during measurements on 27 mast cells in Krebs' fluid with an increased potassium ion concentration (127 mM) was -7 ± 0.38 mV (from -5 to -14 mV). This value differed by a statistically significant degree from that (-11.6 ± 0.48 mV) obtained in mast cells in Krebs' solution of normal salt composition ($P < 0.001$), evidence that depolarization of the mast cell membrane takes place if the extracellular potassium ion concentration is increased.

It was interesting to discover whether depolarization of the mast cell membrane by itself is a trigger mechanism for the liberation of histamine from them. To study this problem the quantity of histamine liberated in solutions of different salt composition was determined. The quantity of histamine liberated spontaneously from mast cells incubated in normal Krebs' fluid was $3.8 \pm 0.63\%$ (16 experiments). The liberation of histamine in depolarizing potassium solution under the same conditions of incubation was $4.1 \pm 0.73\%$, not significantly different from the liberation of histamine in normal Krebs' fluid ($P > 0.5$).

The ability of mast cells kept in depolarizing solution to liberate histamine in response to addition of the histamine-liberating agent was undisturbed. For instance, contact between mast cells and ATP (5 mM) led to liberation of $64.2 \pm 7.35\%$ histamine in normal Krebs' fluid and $73.1 \pm 7.1\%$ in the depolarizing solution. These figures for the histamine-liberating activity of ATP in normal Krebs' solution agree with those obtained by other workers [5]. The liberation of histamine from the mast cells on the addition of ATP was inhibited if calcium ions were absent from the incubating solution. In calcium-free Krebs' solution and in calcium-free depolarizing solution the amount of histamine liberated on the addition of ATP (5 mM) was 7.6 ± 0.74 and $8.4 \pm 0.81\%$, respectively.

The results of these experiments thus show that a resting membrane potential with a maximum of -25 mV (-11.6 ± 0.48 mV) can be recorded in mast cells kept in Krebs' fluid of normal salt composition. The values of the comparatively small membrane potential of the mast cells agree with those obtained for other types of small, inexcitable cells [10, 15] and also for secretory cells such as cells of the salivary [11] and lacrimal glands [9] and the chromaffin cells of the adrenals [6, 12].

Although an increase in the extracellular potassium ion concentration caused depolarization of the mast cell membrane in the present experiments, it did so to only a slight degree. The same feature was observed in the chromaffin cells of the adrenals [12]. By analogy with this type of cells it can be postulated that the low initial level of the membrane potential and the relatively weak influence of the increased extracellular potassium ion concentration on it point to the role of other ions in changing the value of the membrane potential of the mast cells.

It was further shown that depolarization of the mast cell membrane produced by an increase in the extracellular potassium ion concentration by itself did not lead to liberation of histamine from them and the level of histamine liberated spontaneously in Krebs' fluid of normal salt composition and in the solution with an increased potassium ion concentration was the same. Meanwhile the ability of the mast cells to liberate histamine in response to addition of the histamine-liberating agent (ATP) was undisturbed in medium with an increased potassium ion concentration.

These results are in agreement with those obtained for certain secretory cells. It has been shown, for instance, that potassium depolarization of cells of the adrenal cortex does not lead to liberation of steroid hormones from them, while ACTH induces the secretion of these hormones without any appreciable change in the membrane potential of the cells [13].

It can be postulated on the basis of these observations that depolarization of the cell membrane of the mast cells itself, should it arise during their exposure to histamine-liberating substances, does not play a decisive role in triggering the mechanism of histamine secretion.

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