EFFECTS OF *n*-DECYLAMINE AND TOLUIDINE BLUE ON THE ELECTRIC CAPACITANCE OF ISOLATED RAT MAST CELLS

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Abstract—The electric capacitance of isolated rat mast cells and its change under the influence of either *n*-decylamine or toluidine blue was investigated. Since the electric circuit employed in the detection unit is the one merely sensitive for the capacitance changes, output signals pertain to the capacitance of the tested cell alone. *N*-decylamine released histamine without accompanying degranulation; and it caused a marked swelling of mast cells and a striking decrease of capacitance, although electric capacitance is usually proportional to the size of the cell. Morphological changes induced by toluidine blue are seemingly correlated with the changes in capacitance. At the concentrations (25–50 $\mu\text{g/ml}$) in which the mast cell became enlarged, electric capacitances exceeded the control value. However, at the concentrations higher than 100 $\mu\text{g/ml}$ in which the cell became shrunken, the capacitance values were less than control value. Pretreatment with DNP (0.1 mM) or oxyphenbutazone (0.05–0.2 mM) was of little effect in inhibiting the histamine release, morphological alterations and capacitance changes due to *n*-decylamine, but pretreatment with either prevented all of those changes produced by toluidine blue. The mechanism of the capacitance changes in mast cells induced under influence of those compounds is discussed.

Since the early days of electrophysiology, a vast number of investigations have been devoted to the measurement of the membrane potentials and its changes relating to the functional state of the cells. Recent improvements of methodology leading to the voltage clamp technology and intracellular perfusion method advanced our understanding in this field most significantly. On the other hand, electric capacitance of isolated cells has been studied less frequently, although this procedure has an advantage in elucidating the physical properties of the cells (1–4). Falk and Fatt (5) proved that in muscle fiber, the larger component of the capacitance can be related to the inside structure of the cell (the transverse tubular system) rather than cell membranes. The mast cell contained a number of granules surrounded by double-layered membranes which may correspond in some way to the transverse tubular system in the muscle fiber.

It is generally considered that histamine releasing agents may affect the receptive sites on the cell membrane of the rat mast cells and this drug–cell surface interaction triggers a series of events leading to histamine release primarily around the cell membrane (6).

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However, morphological changes in the mast cells exposed to histamine releaser were not invariably dominant in the proximity of the cell membrane, especially when the cells were treated with lower concentrations of histamine releaser (7). A number of events may participate in the process of histamine release in the cytoplasm. To acquire information on what takes place inside isolated rat mast cells exposed to histamine releasers, we measured the electric capacitance in conjunction with that of histamine release and accompanying morphological changes.

MATERIALS AND METHODS

Preparation of mast cells

Wistar rats of either sex, weighing from 300 to 400 g, were stunned and exsanguinated by cutting the carotids. Ten ml of a buffered physiological salt solution [NaCl 154 mM, KCl 2.7 mM, CaCl_2 0.9 mM, Sørensen phosphate buffer 6.7 mM (pH 7.2), and bovine serum albumin 0.05%] was injected into the abdominal cavity and the abdominal wall was gently massaged for 90 sec. The fluid of the abdominal cavity was collected and the mast cells were isolated by gum arabic density gradient centrifugation (8) and resuspended in a small amount of a buffered physiological solution. Mast cells obtained from several rats were pooled to provide a sufficient number of cells for an experiment. In the suspension, mast cells were more than 90% of total cell counts.

Measurement of electric capacitance of isolated mast cells

The electric capacitance of the mast cell was measured with a microcell counter (Toa Denpa, Co., CC-1001) and a hemato-counter (Toa Denpa Co., HT-310). A block diagram of the electric circuit of the detection unit is shown in Fig. 1. Alternating current of 3.5 megacycles per second was supplied to the screen grid (G_2) of the pentode (7737) and this high frequency wave was further transmitted to the control grid (G_1) through a stray capacitance. The G_1 was connected to the tuned circuit through the capacitor and resistor. Phase of the G_1 voltage was shifted to 180° out of phase with that of the G_2 by the capacitor and the tuned circuit. A pair of platinum electrodes, 70 μm in diameter each, were mounted in the detection unit at right angles to a fine pore through which the cells were passed one after another in the stream of the suspending medium provoked by a suction. The electrodes were connected to the tuned circuit. As far as no capacitive change was yielded in the tuned circuit, phase relation between G_1 and G_2 remained as it was, so that no swing in the plate current (I_p) was initiated, and nothing was turned out in output voltage. However, whenever a cell passed through the fine pore of the detection unit, a capacitance change would be brought about between the electrodes and this consequently produces a change in the resonance frequency of the tuned circuit. In consequence, the phases of the G_1 and G_2 potentials become in phase for a short duration and this leads to a swing of I_p , followed by an expeditious voltage output through the pulse transformer. Output voltage was amplified and sent to the discriminator circuit. The G_1 and G_2 actually serve as a phase detector so that an alteration in resonance frequency is prerequisite to producing the output signals; even if a large resistance change takes place between electrodes, this does not permit it to

FIG. 1. Electrical circuit of the detection unit. G_1 : control grid, G_2 : screen grid of the pentode (7737). DU: detection unit, is shown on a larger scale at upper right. Two electrodes (E_1 and E_2) mounted in DU face each other at right angles with a fine pore, and are connected to the tuned circuit. Phase relation between the G_1 , G_2 voltages and the plate current (I_p) is shown in the lower half of the figure. Without a cell between the electrodes, the phases of G_1 and G_2 potentials are out of phase and no current flows in the plate circuit. However, as a cell passes through the pore, a small change in the capacitance between electrodes alters the resonance frequency of the tuned circuit. This results in a slight phase-shift of the G_1 voltage and a current passes in the plate circuit for a short period. The more of in phase between the G_1 and G_2 voltages, the more of the current flow through the plate circuit, and this produces a high output potential.

change the phase and the resulting voltage output. To avoid a contamination of signals generated from other types of cell, such as mononuclear cells and erythrocytes, the input threshold of the discriminator circuit was elevated. The height of the pulses varied in proportion to the magnitude of the electric capacitance of the cell. By increasing the resistance value of the variable resistor in the discriminator circuit (discriminator value), the voltage level at which the pulse is cut off (threshold voltage) becomes higher, so that the number of pulses which can be passed through the circuit was inversely proportionate to the discriminator value. At the discriminator value of 100 the pulses of less than 1 V in the height were eliminated, and at the discriminator value of 1000 the threshold voltage corresponds to 10 V; the relation between threshold voltage and discriminator value was linear within that range. The count displayed at a given discriminator value represents the number of electrical signals passed through a certain input threshold and the measurement was always triplicated, providing a confined distribution. The cell count corresponding to each discriminator value was plotted on the particle size distribution curve. The height of the input signal was digitalized by a pulse-computing circuit (9) and an average of the signals arising from more than 200 cells was exhibited. This value is usually proportional to the cell volume regardless of the cell count in the medium. Because of the difficulty of measuring the actual values of electric capacitance, a ratio of the capacitance values between the control and treated cells was employed as an index of the capacitance

change (relative capacitance).

The pooled mast cells were diluted with the physiological salt solution and divided into 20 samples each containing approximately 5×10^5 mast cells to a final volume of 10 ml. After 10 min of preincubation at 37°C, the histamine releaser, toluidine blue or *n*-decylamine, was added to the medium to make various concentrations, and thereafter incubation was continued for another 5 min. In some experiments, an agent inhibiting mast cell degranulation, such as 2,4-dinitrophenol (DNP) or oxyphenbutazone (10), was added to the medium 5 min prior to the addition of histamine releasers. The final concentration of DNP was fixed to 0.1 mM and in the case of oxyphenbutazone 0.05 mM was employed in the majority of experiments but in a few either 0.1 mM or 0.2 mM was used.

Histamine release from isolated mast cells

The peritoneal mast cell suspensions (1.8 ml) containing 5×10^4 – 5×10^5 cell were pre-warmed at 37°C for 10 min. After the addition of 0.2 ml of either *n*-decylamine (final concentration of 0.5–200 µg/ml) or toluidine blue (final concentration of 0.5–1000 µg/ml), incubation was continued for another 5 min. Thereafter, the histamine releasing process was stopped by chilling the test tubes in ice-water and then centrifuged at 100 g for 10 min at 0°C. On some occasions, 0.5 ml of the suspension was taken for morphological studies before sedimentation. 0.2 ml of 0.1 N HCl was added to the supernatants and the sediments which were resuspended in 2 ml of a fresh buffer solution; and these specimens were placed in boiling water for 5 min. After the samples were neutralized with 0.1 N NaOH, assay for released and residual histamine was done biologically on atropinized guinea pig ileum. As in the case of capacitance measurement, preincubation with DNP or oxyphenbutazone was carried out at corresponding concentrations and the inhibitory effect of these agents upon histamine release was studied.

Morphological studies of mast cells exposed to histamine releasers

After 15 min incubation, the mast cells, with or without fixation by formalin (4%), were transferred to a small glass chamber and morphological changes were observed under an invert-type phase contrast microscope (Olympus PMB, $\times 480$).

Chemicals

Both *n*-decylamine and toluidine blue (Toluidine blue-0) were obtained from E. Merck, Darmstadt. Oxyphenbutazone was kindly provided by Sandoz Co. and 2, 4 dinitrophenol was purchased from Sigma Co. The chemicals used to make the physiological solution were obtained from commercial sources.

RESULTS

Effects of temperature and pH on electric capacitance and particle size distribution curves of isolated mast cells

Electric capacitances and particle size distribution curves of isolated mast cells at different temperatures ranging from 0°C to 40°C are shown in Fig. 2. When the value of electric capacitance at 0°C was used as the standard, the relative capacitances were close

FIG. 2. Effect of temperature on the particle size distribution of isolated rat mast cells. Abscissa: The cell size can be determined as an expedient by discriminator value which actually bounds the cell capacitances. Ordinate: The cell count is represented by the count displayed on the microcell-counter. The accompanying numerals with temperature remarks are normalized capacitance values taking the value at 0°C as unit. Each dot and numeral value in all figures are the means obtained from 3 experiments.

FIG. 3. Effect of pH on the particle size distribution of isolated rat mast cells. The accompanying numerals with pH remarks are the relative capacitances, adopting the value at pH 7.0 as standard.

to 1 at different temperatures. The particle size distribution curves at various temperatures also run close together.

The effects of pH in the suspension medium on electric capacitances of mast cells are shown in the particle size distribution curves of Fig. 3. In this experiment, the values for electric capacitance were standardized taking the value at pH 7.0 as the unit. Distribution curves at different pH's from 4.0 to 10.0 run almost parallel with that of pH 7.0 and within a fairly narrow range. With decreasing capacitance, the cell counts on each distribution curve also decreased.

*Effect of *n*-decylamine*

Morphological changes in isolated mast cells treated with various concentrations of *n*-decylamine were investigated in relation to histamine release and capacitance changes. One striking change in morphology was a marked swelling of the cells and this was observed at the concentrations ranging from 5 $\mu\text{g/ml}$ to 200 $\mu\text{g/ml}$. As shown in Fig. 4, the most marked swelling was evident with a concentration of 25 $\mu\text{g/ml}$. At higher concentrations the swelling was less marked. At every concentration tested, the cytoplasmic granules were enlarged but no degranulation was observed. Neither DNP nor oxyphenbutazone, even

FIG. 4. Morphological changes of isolated mast cells induced by different concentrations of *n*-decylamine. a: control; b: 5 $\mu\text{g/ml}$; c: 25 $\mu\text{g/ml}$ and d: 200 $\mu\text{g/ml}$. At each concentration, mast cells were exposed for 15 min at 37°C. The most remarkable swelling of the cell was observed at 25 $\mu\text{g/ml}$ (all $\times 400$).

FIG. 5. Histamine release from isolated rat mast cells exposed to various concentrations of *n*-decylamine with or without pretreatment by either DNP or oxyphenbutazone. Histamine release due to *n*-decylamine alone (●) was initiated at 5 $\mu\text{g/ml}$ and reached the maximum at 50 $\mu\text{g/ml}$. Pretreatment with DNP (0.1 mM, $\triangle$), or oxyphenbutazone (0.2 mM, $\square$) was of little effect.

in 0.2 mM, had any preventive effect on the morphological changes induced by *n*-decylamine.

Histamine releases from mast cells under the effect of various concentrations of *n*-decylamine are shown in Fig. 5. The least histamine release was provoked at 5 $\mu\text{g/ml}$ and the maximum release, about the level of 94%, was achieved at 50 $\mu\text{g/ml}$, and this level was maintained at higher concentrations. No preventive effect of DNP nor oxyphenbutazone (0.2 mM) on histamine release induced by different concentrations of *n*-decylamine was observed.

Fig. 6 shows the particle size distribution curves and capacitance changes under the effect of various concentrations of *n*-decylamine. The slope of the distribution curves

FIG. 6. Effect of *n*-decylamine on the particle size distribution of isolated rat mast cells. The numerals abreast with the concentration remarks were relative capacitances of isolated mast cells exposed to the corresponding concentrations of histamine releasers in this and the following figures. In the case of *n*-decylamine, the mast cell capacitance decreased in reverse proportion to an increase of *n*-decylamine concentration in the medium.

FIG. 7. Effect of toluidine blue on the particle size distribution of isolated rat mast cells. At lower concentrations (20 to 100 $\mu\text{g/ml}$) the capacitance of the cell was greater than that of the control and this was reflected in the size distribution curves which run above the control curve. At higher concentrations (more than 500 $\mu\text{g/ml}$) all values depreciated.

became steeper with increasing concentration. In conjunction with this, the ratio of electric capacitance decreased reciprocally with an increase of the concentration, although cell volume increase is usually accompanied by an increase of electric capacitance (11). Little difference in the relative capacitances of the mast cells exposed to 200 $\mu\text{g/ml}$ of *n*-decylamine was noted with or without pretreatment of either DNP or oxyphenbutazone (0.2 mM).

Effect of toluidine blue

In concentrations between 10 and 50 $\mu\text{g/ml}$, mast cells revealed a marked swelling with conspicuous degranulation in most cells and the distinctly swollen granules were easily discernible. While at 100 $\mu\text{g/ml}$ the cell surface showed remarkable indentations with a number of small protrusions. In concentrations higher than 100 $\mu\text{g/ml}$ the affected cells shrank to a size smaller than control cells and appeared with smooth cell surfaces. With an increase of the concentration (up to the maximum of 1000 $\mu\text{g/ml}$), a decrease in the cell volumes became evident. These observations were in agreement with the findings reported by Yamasaki and Komoto (12). The relative capacitances were 1.35 and 1.15 at the con-

centrations of 20 and 50 $\mu\text{g/ml}$, respectively (Fig. 7). At concentrations higher than 100 $\mu\text{g/ml}$ there was a significant decrease in cell size, and the capacitances were less than 1. The particle size distribution curve reflects changes in electric capacitance. At the higher concentrations of more than 500 $\mu\text{g/ml}$, few counts were displayed in near maximum or maximum discriminator values. While in the lower concentrations the cell counts in the medium exceeded the corresponding control counts in each of the discriminator values.

Pretreatment with either DNP or oxyphenbutazone (0.05 mM) was highly effective in preventing the changes in capacitance. The relative capacitances of the affected cells were all in close proximity to the control value. All the distribution curves ran closely together, intersected each other and the cell counts at each discriminator value were clustered around the control with little deviation.

Toluidine blue produced a detectable release of histamine from mast cells at 5 $\mu\text{g/ml}$, which increased with higher concentrations of toluidine blue. The maximum release of 64% was achieved in the range of 50 to 100 $\mu\text{g/ml}$ and at higher concentrations (250 to 1000 $\mu\text{g/ml}$) histamine release declined. In contrast with *n*-decylamine, pretreatment with either DNP or oxyphenbutazone (0.1 mM) caused almost complete inhibition of the histamine release by toluidine blue, nearly full prevention being attained even in the release provoked at 1000 $\mu\text{g/ml}$.

DISCUSSION

It has been generally considered that an interaction between the mast cell and a histamine releaser takes place at the cell surface and this initiates a series of events leading to histamine release (6, 13). Information concerning the initial changes occurring at the cell membrane can be obtained from the measurement of the physical properties of the cell membrane (14, 15). However, a number of events occurring on the inside of the cell may be involved in the process to complete the histamine release, so that any information from the inside of the mast cell may be contributory in elucidating the mechanism of histamine release.

It has been mentioned that the dielectric constant of a NaCl solution at 25°C (ϵ_c) can be calculated as $\epsilon_c = \epsilon_w - 2c\delta$, in which ϵ_w —dielectric constant of water at 25°C (78.54); δ —molar diminution of the dielectric constant (for a NaCl solution, this is -5.5); c —molar concentration of NaCl. In 0.15 M NaCl solution, ϵ_c of 76.89 can be obtained (16). Schwan and Cole (17) found that a dielectric constant of the muscular tissue at the 3.5 MHz attained the value of about 1000. If the dielectric constant of mast cells bears a close resemblance to this value, capacitance change taking place between the electrodes as a mast cell passes through the pore may be large enough as a source of the signal.

Incubation of mast cells with 50 $\mu\text{g/ml}$ of *n*-decylamine released histamine by 94% in the present experiment and this coincides well with the result submitted by Bloom and Haegermark (18) (histamine release of 93%). In many instances, the capacitance of the cell is proportional to the cell volume in the physiological condition (11). However, *n*-decylamine caused a marked decrease of capacitance, even though the cell volume increased remarkably. In association with this, Cole (11) reported the membrane capacity of hippocampal

eggs decreased as a linear function of the surface area when eggs were swollen in diluted sea water, interpreting this as being a result of a constitutional change in the membrane. The finding in the present experiment coincides with electron microscopic observations (18) that *n*-decylamine disrupts not only the cytoplasmic but also the perigranular membranes so as to give a fragmented appearance in each, and most of the granules were swollen and grossly altered exhibiting a coarse and low electron density. Impairing the membrane necessitates an intrusion of extracellular fluid into the cytoplasm and this results in subsequent increase of the cell volume. The capacitance of the cell membrane has been measured in a variety of cells (1-4). Falk and Fatt (5) reported that the muscle fiber has two capacitative channels. The smaller value probably represents the capacity of the surface membrane and the larger capacity may arise from the inside of the cell owing to the transverse tubular system which is a complicated invagination of the cell membrane. After a selective disruption of the transverse tubular system, the capacitance of the muscle fiber decreased remarkably (19). It has been reported that the mast cell is filled with a large number of granules and the perigranular membrane consists of a double-layered membrane (20) as is the case in most cell membranes, and that mast cell granules may be retained within invaginations of the plasma membrane (21). From the analogy between the perigranular membrane in the mast cell and the transverse tubular system in the muscle fiber, it may be assumed that the mast cell has a considerably high capacitance which is largely contributed by numerous perigranular membranes. *N*-decylamine destroys the perigranular membrane even in a low concentration such as 5 $\mu\text{g/ml}$ (18). This is probably the reason for a significant reduction of relative capacitance in such a low concentration. With an increase of the concentration, the morphological changes were aggravated and this further decreased the mast cell capacitance. A severe paucity of organized membrane and derangement of the inside structure account for the capacitance decrease. The membrane destroying property of *n*-decylamine (18) explains the failure of both DNP and oxyphenbutazone in inhibiting histamine release as well as capacitance changes of the mast cells affected with *n*-decylamine. These two substances are competent in obstructing histamine release caused by many other releasers (10).

Morphological changes elicited by toluidine blue are more complicated than those of *n*-decylamine as mentioned previously. It has been observed that in the concentrations between 10 and 50 $\mu\text{g/ml}$ toluidine blue induces the extrusion of some granules from isolated mast cells with simultaneous enlargement of the cells, and the swollen granules constitute the majority of the granules in the cytoplasm. The capacitance changes of mast cells exposed to toluidine blue seem to run parallel to the changes in the cell size. One of the characteristics in the mast cell morphology responding to lower concentrations of toluidine blue is a marked degranulation. In relation to this, it has been reported (13) that the local degranulation of the mast cell can be provoked more than a few times in a single cell by repeating the restricted applications of basic histamine releasers to different spots on the cell surface. This finding suggests that the extrusion of granules does not necessarily mean the disruption of the cell membrane at the degranulated sites. If the cell membrane loses

continuity at the degranulated site, then this inevitably brings about an entry of extracellular fluid into the cytoplasm and may damage the cell so that no further degranulation will follow. On electron microscopic observation, toluidine blue does not disrupt the perigranular membrane at the concentration of 50 $\mu\text{g/ml}$, although some morphological changes were observed such as swelling and alteration of the granules (7). However, at 300 $\mu\text{g/ml}$ toluidine blue provoked drastic morphological changes in the affected cell. Several or more of the perigranular membranes fused to each other losing their own constructions, and severely altered granules were contained in a resulting large cavity. Nearly half of the cytoplasm was occupied by those cavities (22). This clearly indicates that the remarkable decrease of the capacitance brought about at the higher concentrations of toluidine blue is largely due to the destruction of the main source of the capacitance, as in the case of *n*-decylamine, but some can be accounted for by the shrinkage of the cell. Both DNP and oxyphenbutazone almost completely impede the morphological changes, and histamine release as well as capacitance changes, caused by toluidine blue.

It has been shown in the present experiment that the measurement of mast cell capacitance is capable of bringing out some information regarding histamine release from the inside of the cells. However, there exist some imperfections in the instrumentation, so that electric capacitance had to be expressed as a relative value instead of the absolute value. The resolution of this problem is left for the future.

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