IONIC MECHANISM OF THE RESTING MEMBRANE DEPOLARIZATION OF FROG SARTORIUS MUSCLE INDUCED BY DIMORPHOLAMINE

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Accepted November 22, 1979

Abstract—Ionic mechanism of the resting membrane depolarization of frog sartorius muscle induced by dimorpholamine was studied in a variety of ionic conditions. The resting membrane was depolarized in a dose-dependent manner reaching a maximum with a dose of $6 \times 10^{-5}$ M. The amount of the depolarization (1.4 mV) was suppressed with doses over $10^{-4}$ M. This dose-dependent response was accompanied by change in the membrane conductance. The chloride conductance was increased by 16% and the potassium conductance by 7%, however the sodium conductance did not significantly change with a dose of $2 \times 10^{-5}$ M. The slight depolarization induced by the drug was well explained by the changes in the ionic conductance. The effects of these actions on the membrane action potentials were simulated, suggesting that this drug possesses the nature of a stimulant on excitable membranes, yet does not produce any appreciable depolarization blockade.

Dimorpholamine depolarizes the resting membrane potential of frog sartorius muscle and the degree of depolarization is slight even in the case of high doses (1). This finding is of considerable interest since a slight depolarization may increase the excitability of the membrane without producing a significant depolarization block (2, 3).

In the present work, the ionic mechanism of dimorpholamine on the resting membrane depolarization was studied using electrophysiological techniques. Changes in membrane conductance with dimorpholamine application were measured in a variety of ionic conditions. The slight depolarization induced by the drug was well explained by the changes in the ionic conductance, and the effects of these actions on the membrane action potentials were simulated with the aid of a computer.

MATERIALS AND METHODS

Materials: Sartorius muscles of Rana nigromaculata were carefully isolated and mounted on a block of Perspex lying in a small bath (1 ml) at 20°C.

Measurements of membrane potential and conductance: Resting potentials were recorded with a 2M K-acetate microelectrode of 10–15 MΩ. A coarse microelectrode filled with 3M KCl was used as a reference electrode.

The membrane conductance ($g_m$) was measured with the two microelectrodes inserted close together into the same fiber (100 μm away), one to record the membrane potential and the other to pass current through the membrane (4). For passing current, 2M K-citrate
microelectrodes with resistances of 5–10 MΩ were used. Hyperpolarizing or depolarizing current pulses of 700–1000 msec duration and intensities up to about 5 × 10⁻⁸ A (I) were used, and the electrotonic potentials at the end of the current pulse (V) were measured. The results have been presented as plots of the membrane current density (I_m) required to produce a uniform change in potential (V_m) over the whole fiber to facilitate comparison among the different fibers using the relation:

\[ I_m = \frac{R_i}{\pi d^2} \cdot I \cdot \frac{dI}{dV_m} \]  

(1)

where \( R_i \) represents the specific resistance of the myoplasm, and \( d \), diameter of the muscle fiber (5, 6). Changes in \( g_m \) are then directly proportional to changes in the current density producing a given voltage displacement. To study membrane characteristics near the resting potential, the muscle membrane was always held at about −95 mV by injecting a DC current. The membrane potential at rest was monitored by cutting off the DC current for a moment, or extrapolating the voltage to zero holding current in the current-voltage relation when necessary. The liquid junction potential in the potential recording was corrected using the Henderson’s equation (7).

Solutions and chemicals: Normal Ringer was composed of (mM): Na⁺, 120.15; K⁺, 2.5; Ca⁴⁺, 1.8; Cl⁻, 121.1; HPO₄⁻, 2.15; H₂PO₄⁻, 0.85 with pH of 7.2. Methylsulphate was used as an impermeable anion (8) replacing chloride in "K-Ringer". For "Cl-Ringer", K was replaced with Na. Both K and Cl were substituted for Na and methylsulphate in "Na-Ringer".

Crystalline dimorpholamine (Eisai, Tokyo) was used. Tetrodotoxin (TTX) was used in a concentration of 2 × 10⁻⁷ M to prevent muscle movement and d-tubocurarine 2 × 10⁻⁶ M to exclude a possible effect on the end-plate. The experiments were performed at 20°C.

RESULTS

Dose-response relation in resting membrane depolarization: Changes in membrane potential were measured 5 min after application of the drug, in which 10 measurements were made in one muscle. Figure 1 shows the dose-response curve from 5 muscles in each point. There was a dose-dependent increase in depolarization, but with a slight inhibition in doses over 10⁻⁴ M. The extent of depolarization was about 1.4 mV with a dose of 2 × 10⁻⁵ M. Essentially the same results were obtained with TTX 2 × 10⁻⁷ M and d-tubocurarine 2 × 10⁻⁶ M.

Dose-response relation in membrane conductance: Potential change produced by a constant current was measured before, during, and after application of dimorpholamine, and the slope conductance at a resting potential was calculated from the current-voltage relation with the aid of regression curve fitting to ensure the steady-state electrotonic potential produced by a weak constant pulse of current (cf. 5). Changes (\%) in \( g_m \) are illustrated as a function of drug concentration in Fig. 2. The membrane conductance also showed a dose-dependent increase with a slight inhibition with high doses. Almost the same con-
ductance change occurred with application of $2 \times 10^{-7}$ M TTX. All experiments described below were therefore carried out in each solution containing TTX to prevent possible movement of the muscle tissue.

**Effect of dimorpholamine on $g_m$ in Na-Ringer:** The microelectrodes were inserted into

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**FIG. 1.** Effect of dimorpholamine on membrane depolarization. Ordinate: membrane potential in mV. Abscissa: drug concentration in M. Each symbol is mean ± S.E. of 5 experiments.

**FIG. 2.** Effect of dimorpholamine on membrane conductance. Each value shows the average of 5 experiments. Ordinate: membrane conductance in % change. Abscissa: drug concentration in M.

**FIG. 3.** Effect of dimorpholamine on the current-voltage relation in Na-Ringer. Ordinate: membrane current in A/cm². Abscissa: membrane potential in mV. Filled circles, control in Na-Ringer; filled squares, dimorpholamine $2 \times 10^{-5}$ M in Na-Ringer; filled triangles, after returning to normal Ringer.

**FIG. 4.** Effect of dimorpholamine on the current-voltage relation in Cl-Ringer. Ordinate: membrane current in A/cm². Abscissa: membrane potential in mV. Filled circles, control in Cl-Ringer; filled squares, dimorpholamine $2 \times 10^{-5}$ M in Cl-Ringer; filled triangles, after returning to normal Ringer.
muscle fibers equilibrated in the Na-Ringer and $g_m$ change by application of the drug was measured after the stability of the membrane was attained, 50-60 min after potassium and chloride were removed (Fig. 3). Administration of dimorpholamine $2 \times 10^{-5}$ M produced little change in $g_m$ (Fig. 3).

Replacement of potassium and chloride with sodium and methylsulphate produced a considerable $g_m$ increase on readmission of those ions after the measurement of the drug action (triangle in Fig. 3).

**Effect of dimorpholamine on membrane conductance in Cl-Ringer:** Since no significant change in the sodium conductance in the resting membrane ($g_{Na}$) was observed with the drug, sodium was used as a substitute for potassium when the effect on the chloride conductance ($g_{Cl}$) was determined. A membrane current-voltage relation in the Cl-Ringer is given in Fig. 4. The $g_{Cl}$ of the resting membrane ($-95$ mV) was $40 \pm 2.1 \mu$mho/cm$^2$ on the average of 5 muscles where $145 \Omega\text{cm}$ for $R_i$ was used in Eq. (1). Dimorpholamine ($2 \times 10^{-5}$ M) increased the $g_{Cl}$ by an average of $16\%$ (Fig. 4, Table 1). The effect of potassium

| Chloride conductance | Potassium conductance | Sodium conductance |
|----------------------|-----------------------|-------------------|
| 15.7 ± 3.8           | 7.1 ± 1.2             | 1.5 ± 3.2         |
| (n = 7)              | (n = 5)               | (n = 5)           |

For calculation see text. The change was significant for the chloride and potassium conductances with $t$-test ($P < 0.001$).

![Fig. 5. Effect of dimorpholamine on the current-voltage relation in K-Ringer.](image)

Ordinate: membrane current in A/cm$^2$. Abscissa: membrane potential in mV. Filled circles, control in K-Ringer; filled squares, dimorpholamine $2 \times 10^{-5}$ M in K-Ringer; filled triangles, after returning to normal Ringer.
removal was also confirmed by observing the \( g_m \) increase on readmission of the ion (triangle in Fig. 4).

**Effect of dimorpholamine on potassium conductance in K-Ringer:** All the chloride was replaced with methylsulphate and the microelectrodes were inserted after the equilibration of the muscle fiber in the solution. The \( g_K \) of the resting membrane \((-95 \text{ mV})\) was \( 83 \pm 12.0 \mu \text{mho/cm}^2 \) on the average of 7 muscles with the same \( R_i \) value as above. The \( g_K \) slightly increased by 7% on the average during exposure to the drug, \( 2 \times 10^{-5} \text{ M} \) (Fig. 5, Table 1). On readmission of chloride, the \( g_m \) greatly increased (triangle in Fig. 5).

**DISCUSSION**

The present study clearly demonstrates that dimorpholamine raises the resting membrane conductance for chloride and potassium ions (Table 1). Taking into account that the ionic fractions of the total membrane conductance were approximately 0.33 and 0.67 for \( g_{cl} \) and \( g_K \), respectively, (Figs. 4 and 5), 16% and 7% increases in \( g_{cl} \) and \( g_K \) by the drug \( (2 \times 10^{-5} \text{ M}) \) were in a fairly good agreement with the 10% increase in the resting membrane conductance observed in normal Ringer's solution (Fig. 2).

From the \( g_{cl} \) and \( g_K \), measured at the resting membrane at \(-95.3 \text{ mV}\) (cf. Figs. 4 and 5), \( P_{cl} \) and \( P_K \) were estimated to be \( 0.85 \times 10^{-6} \) and \( 1.99 \times 10^{-6} \text{ cm/sec} \), respectively, using the constant field equation (9-11). Incorporating these permeabilities into the equation, \( P_{Na} \) to give \(-95.3 \text{ mV}\) was found to be \( 5 \times 10^{-9} \text{ cm/sec} \). Therefore, 1.5% increase in \( g_{Na} \), even if present (Table 1), should not create any significant potential change. In this calculation,

| Table 2. Values of parameters used in the computer simulation |
|-------------------------------------------------------------|
| Parameter | Value |
| Temperature | 20°C |
| Membrane capacity | 1.69 \( \mu \text{F/cm}^2 \) |
| \( \alpha_m \) | 0.128/msec |
| \( \beta_m \) | 1.28/msec |
| \( V_m \) | -42 mV |
| \( g_{Na} \) | 117 \( \mu \text{mho/cm}^2 \) |
| \( V_{Na} \) | 50 mV |
| \( \alpha_h \) | 0.0096/msec |
| \( \beta_h \) | 2.081/msec |
| \( V_h \) | -41 mV |
| \( \alpha_r \) | 0.01409/msec |
| \( \beta_r \) | 0.05917/msec |
| \( V_r \) | -40 mV |
| \( g_K \) | 33 \( \mu \text{mho/cm}^2 \) |
| \( V_K \) | -101 mV |
| \( g_L \) | 0.3 \( \mu \text{mho/cm}^2 \) |
| \( V_L \) | -93 mV |

The equations in Table 6A by Adrian et al. (1970) were used for the rate constants as a function of the membrane potential. The \( Q_{st} \) of the rate constant was assumed to be 2.5, and for \( g_{Na} \) and \( g_{K} \) it was taken as 1.5. *The membrane capacity determined by Sevcik and Narahashi (1972) on the sartorius muscle treated with ethylene glycol was used.
activity coefficients of all the ions were assumed to be of the same value in Ringer's solution and the myoplasm for simplicity. The intracellular ionic concentrations used were 137, 3.6, and 15 mM for potassium, chloride and sodium respectively (7, 11, 12, 13).

Since the chloride equilibrium potential ($E_{Cl}$) of frog sartorius muscle is positive to the resting potential (12-14), the concomitant permeability increase in chloride and potassium ions by the drug would be linked to the depolarization of the resting membrane, which could not go beyond $E_{Cl}$. This ionic mechanism may be beneficial for this drug to act as a stimulant on the excitable membranes because it would not induce a significant depolarization blockade.

Probable effects of the membrane depolarization (2 mV) and conductance increase (10%) on behaviour of the excitable membrane were simulated using the kinetic model developed by Adrian et al. (1970) for the frog sartorius muscle (15). In this simulation, the other effects by this drug (1) were omitted for simplicity. The integration procedures were the same as shown in our previous study (3). The constants used are given in Table 2. The simulation was performed on the muscle membrane decoupled by treating with ethylene glycol (16) and space-clamped for simplicity.

When stimulating current was increased from $-305$ to $-325 \mu A/cm^2$, the larger currents elicited action potentials. (Fig. 6A-a, b, c). The same amount of stimuli was added to the same muscle fiber, as affected by dimorphlamine, $2 \times 10^{-5}$ M. The same smallest stimulus

![Fig. 6. Computer simulation of membrane action potential as affected by dimorphlamine. Ordinate: membrane potential in mV. Abscissa: time in msec. Upper, control, Lower, dimorphlamine $2 \times 10^{-5}$ M. $g_L$ was increased by 10% and the resting potential was depolarized by 2 mV. Stimulation: $-325 \mu A/cm^2$ for 0.2 msec (a), $-315 \mu A/cm^2$ (b), and $-305 \mu A/cm^2$ (c).]
$-305 \text{ } \mu \text{A/cm}^2$, which failed to generate action potential in control (Fig. 6A), could elicit action potential with a higher rate of rise under the influence of the drug (Fig. 6B-c). The action potential returned to the original level more rapidly in the treated muscle fiber than in the control. Dimorpholamine may induce a state of stability but one of being ready to fire, once the muscle membrane is stimulated.

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