*PERMEABILITY AND INTERNAL CONCENTRATION OF IONS DURING DEPOLARIZATION OF THE ELECTROPLAX**

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Communicated by David Nachmansohn, July 19, 1967

Electroplax from the electric organs of *Electrophorus electricus* are large, sheet-like cells derived from muscle, whose function, the generation of transcellular current, is a consequence of their asymmetry: the membrane on one side of the cell is innervated and electrically and chemically excitable, while that on the other side is noninnervated and inexcitable.[1, 2] The application of acetylcholine and related compounds to the innervated membrane results in a depolarization of the cell that is most likely initially due, as in muscle,[3] to changes in the permeability of the subsynaptic regions of the membrane to Na^+ and K^+.[4–6] This depolarization, measured across the innervated membrane after a 1–20-minute application of a depolarizing agent, has been taken as a measure of the primary response of the electroplax to such agents, and inferences regarding the acetylcholine receptor have been drawn from dose-response data so obtained.[7–9] This depolarization is not, however, simply and solely a function of the initial permeability changes. During these depolarizations, which are relatively long compared with the neurally evoked postsynaptic potential of 1–2-millisecond duration, there appear to be appreciable changes in the intracellular concentrations of Na^+ and K^+. These concentration changes, and probably in addition the compensating process of active transport, partially determine the potential difference across the membrane. First, evidence will be presented indicating that the concentration changes are appreciable, and, second, these changes and the permeability changes will be estimated. The estimate of the permeability change should provide a better measure of the primary response of the electroplax to acetylcholine and its congeners.

Methods.—Electroplax were isolated[10] and were mounted in a Lucite holder in which the cell separates two pools of a modified Ringer's solution (containing 165 mM NaCl, 2.3 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 1.2 mM K_2HPO_4, 0.3 mM KH_2PO_4, 10 mM glucose, pH 7.1). The holder used (details will appear elsewhere) was a modification of that described previously[11] in that the electroplax is placed horizontally, innervated side down, and part of the innervated membrane, exposed through a window (3×0.75 mm), is in contact with a stream of fresh solution. The rate of flow was such that the solution bathing the innervated side could be changed in a few seconds. Potential differences across either membrane were measured between an intracellular glass microelectrode filled with 3 *M* KCl and agar bridges in the outside solutions.

Results and Discussion.—Effect of ouabain on the repolarization: Normal depolarizations of both the innervated and the noninnervated membranes in response to 40 μM carbamylcholine on the innervated side are shown in Figure 1*A*. Washing the carbamylcholine out with Ringer's solution causes a rapid repolarization (with an overshoot). In contrast, the repolarization following prior exposure of the electroplax to ouabain is strongly inhibited (Fig. 1*B*). Ouabain is believed to be a

specific inhibitor of the energized extrusion of Na^+, to which is coupled, either directly or indirectly, the uptake of K^+.[12] Whereas carbamylcholine acts only on the innervated membrane, ouabain inhibits the repolarization more strongly when added to the noninnervated side than to the innervated side. After exposure to ouabain, the repolarization following small as well as large depolarizations is inhibited, and the extent of inhibition increases with the number of depolarizations and the time of exposure to ouabain. Twenty minutes after the application of 10^{-4} *M* ouabain to the noninnervated membrane, the repolarization following only 30 seconds of 20 μM carbamylcholine was inhibited. In contrast, after 60 minutes, 10^{-4} *M* ouabain had, in the absence of induced depolarizations, only a slight effect on the resting potential and action potential. The effects of ouabain implicate active Na^+ transport in the repolarization process and, hence, indicate a significant accumulation of Na^+ and loss of K^+ (see below) during the depolarization. Previously, N-ethylmaleimide was found to inhibit the repolarization of the electroplax, and this effect was also probably due to inhibition of ATP formation or of its utilization by the Na^+ pump.[9]

Analysis of the depolarization: Both the potential difference measured across the innervated membrane, ψ_I (outside *minus* inside), and that measured across the noninnervated membrane, ψ_N (outside *minus* inside), decrease following application of carbamylcholine to the innervated membrane (Fig. 1). The decrease in

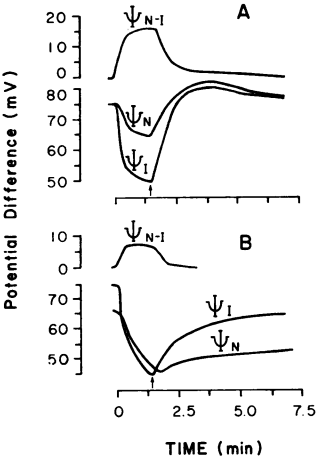

FIG. 1.—Response of the electroplax to 40 μM carbamylcholine.

The effect of carbamylcholine on the transcellular potential difference (ψ_{N-I}), the p.d. across the innervated membrane (ψ_I), and the p.d. across the noninnervated membrane (ψ_N) were measured consecutively. The traces have been superimposed on the time axis; carbamylcholine was added to the innervated membrane in each case at 0 min and washed out at the arrow.

(*A*) Control.

(*B*) 10^{-4} *M* ouabain was present on the noninnervated side of the cell for 10 in before ψ_I was measured, 25 min before ψ_N was measured, and 40 min before ψ_{N-I} was measured.

ψ_I may be due, in part, to a permeability change *per se*, but the decrease in ψ_N is unlikely to be so caused, since presumably the permeability of the noninnervated membrane does not change: carbamylcholine does not reach it, and, in any case, carbamylcholine does not act on the noninnervated side. Furthermore, transcellular current driven by the change in ψ_I, and returning around the cell past the window, could have only a negligible effect on ψ_N since the resistance of the noninnervated membrane is only $^1/_{30}$ of the resistance of the innervated membrane.[2] Therefore, the depolarization of the noninnervated membrane is most likely due to a change in intracellular ion concentration, namely, a decrease in (K^+). ψ_N decreases to a lesser extent than ψ_I, and hence, in the presence of carbamylcholine the transcellular potential, $\psi_{N-I} = \psi_N - \psi_I$, is nonzero (Fig. 1).

Expressions for the changes in ψ_I, ψ_N, and ψ_{N-I} may be derived from the Goldman equation,[13]

$$\psi = \frac{RT}{F} \ln \frac{K_i + \rho Na_i + \sigma Cl_o}{K_o + \rho Na_o + \sigma Cl_i}, \quad (1)$$

where ψ is outside potential *minus* inside potential, i and o designate intra- and extracellular concentrations, ρ is the ratio of Na^+ permeability to K^+ permeability, and σ is the ratio of Cl^- permeability to K^+ permeability. The Cl^- permeability has been said to be low in the electroplax.[6, 14, 15] I have observed that the immediate effect of changing from the usual outside solution (given above), containing 175 mM Cl^-, to solutions in which most of the Cl^- had been replaced by SO_4^{2-} or by glutamate ((Cl^-) = 10–20 mM) was a transient depolarization of the innervated membrane by 1–2 mV and of the noninnervated membrane by 3–5 mV. These results imply that σ is less than 0.1 for the innervated membrane and less than 0.2 for the noninnervated membrane.[16] Because of the relative magnitude of the terms in equation (1), Cl^- may be neglected with a resulting error of less than 10 per cent. Equation (1) reduces thereby to

$$\psi = \frac{RT}{F} \ln \frac{K_i + \rho Na_i}{K_o + \rho Na_o}, \quad (2)$$

which I will assume applies to both membranes with the same resting value of ρ. Now let primed variables represent values at a given time after addition of a depolarizer and unprimed variables the values before, and let $\Delta\psi = \psi' - \psi$, then the positive extents of depolarization of the two membranes are

$$-\Delta\psi_I = \frac{RT}{F} \ln \frac{(K_i + \rho Na_i)(K_o + \rho' Na_o)}{(K_i' + \rho' Na_i')(K_o + \rho Na_o)} \quad (3)$$

and

$$-\Delta\psi_N = \frac{RT}{F} \ln \frac{K_i + \rho Na_i}{K_i' + \rho Na_i'}. \quad (4)$$

Furthermore, since $\Delta\psi_{N-I} = \Delta\psi_N - \Delta\psi_I$ (which is positive),

$$\Delta\psi_{N-I} = \frac{RT}{F} \ln \frac{(K_i' + \rho Na_i')(K_o + \rho' Na_o)}{(K_i' + \rho' Na_i')(K_o + \rho Na_o)}. \quad (5)$$

Equation (4) expresses mathematically the argument given above that the depolarization of the noninnervated membrane implies a significant change in intracellular Na^+ and K^+ concentrations. Also, consistent with this interpretation is the fact that, after ouabain, $-\Delta\psi_N$ increases (Fig. 1); i.e., there is greater net accumulation of Na^+ and net loss of K^+ when the Na^+ pump is inhibited. Prior exposure to ouabain also increases $-\Delta\psi_I$, but less than $-\Delta\psi_N$, and decreases $\Delta\psi_{N-I}$. These effects are in agreement with equations (3) and (5).

Expressions relating ρ', Na_i', and K_i' to the measured potential differences and and to ρ, Na_i, K_i, Na_o, and K_o may now be derived from the above equations. ρ is not known but may be estimated from equation (2). Mean values for K_i and Na_i have been determined to be 170 mM and 10 mM, respectively.[4, 14] The mean

resting potential of 25 cells was 81 mV. Inserting these values, and RT/F = 25 mV, K_o = 5 mM, Na_o = 165 mM, into equation (2) and solving for ρ yields ρ = 0.01, a value reasonably similar to estimated values of ρ for other cells.[17-19] Assuming that ρ is constant, we may calculate K_i for each particular cell from equation (2) in the form

$$K_i = \xi(K_o + \rho Na_o), \tag{6}$$

where ρNa_i has been neglected and $\xi = e^{(F\psi/RT)}$ ($\psi = \psi_I = \psi_N$). As a consequence of the relatively low permeability of Cl^-, it may be assumed that the sum of the intracellular concentrations of Na^+ and K^+ is constant (this also follows if nearly all the impermeable intracellular solutes are univalent anions);[18] i.e.,

$$Na_i + K_i = Na_i' + K_i' = M \tag{7}$$

and inserting the mean values given above, M = 180 mM. Combining equations (4) and (7) we get an expression for K_i',

$$K_i' = \frac{K_i(1 - \rho) + \rho M(1 - \Delta\xi_N)}{\Delta\xi_N(1 - \rho)}, \tag{8}$$

where $\Delta\xi_N = e^{(-F\Delta\psi_N/RT)}$.

Finally, solving equation (5) for ρ', we obtain

$$\rho' = \frac{\Delta\xi_{N-I}K_i'(K_o + \rho Na_o) - K_o(K_i' + \rho Na_i')}{Na_o(K_i' + \rho Na_i') - \Delta\xi_{N-I}Na_i'(K_o + \rho Na_o)}, \tag{9}$$

where $\Delta\xi_{N-I} = e^{(F\Delta\psi_{N-I}/RT)}$. Equations (6), (7), (8), and (9) may be combined to yield ρ' as a function of fixed parameters and measured variables,

$$\rho' = \frac{A\Delta\xi_{N-I}[A(1 - \rho)\xi + \rho M(1 - \Delta\xi_N)] - K_o(1 - \rho)[A(1 - \rho)\xi + \rho M]}{Na_o(1 - \rho)[A(1 - \rho)\xi + \rho M] - A\Delta\xi_{N-I}[M(\Delta\xi_N - \rho) - A(1 - \rho)\xi]}, \tag{10}$$

where $A = K_o + \rho Na_o$. An approximate form of the above equation shows clearly the principal dependence of ρ' on $\Delta\xi_{N-I}$ (i.e., on $\Delta\psi_{N-I}$), and vice versa,

$$\rho' - \rho \cong \left(\frac{K_o}{Na_o} + \rho\right)(\Delta\xi_{N-I} - 1), \tag{11}$$

which is valid for $\rho Na_i'/K_i'$ much less than 1 and $Na_o/(K_o + \rho Na_o)$ much greater than $\Delta\xi_{N-I}Na_i'/K_i'$. The first condition is satisfied over the entire range of responses, but the second is well satisfied for responses about half maximal and less and only during the initial part of larger responses.

As an example, these equations will be applied to the data represented in Figure 1*A*. The effect of carbamylcholine on each of the potential differences was measured consecutively on the same cell with a 10–20-minute recovery between responses; ideally, the changes should be measured simultaneously (only two of the three have to be measured). However, normally (and in this case) the cell repolarizes to the initial resting potential following a depolarization, and the curve obtained by subtracting ψ_I from ψ_N is superimposable upon the measured trace of ψ_{N-I}. (Neither condition applies to the data in Figure 1*B* obtained in the presence

of ouabain, which prevents the cell from returning to its initial state.) The assumed fixed values are $\rho = 0.01$ and $M = 180$ mM; also, $Na_o = 165$ mM and $K_o = 5$ mM. Initially, $\psi = \psi_I = \psi_N = 77$ mV, and equations (6) and (7) yield the initial values, $K_i = 145$ mM, $Na_i = 35$ mM. Seventy-five seconds after the addition of 40 μM carbamylcholine, $-\Delta\psi_N = 12.5$ mV and $\Delta\psi_{N-I} = 16$ mV. Equation (8) yields $K_i' = 88$ mM, and then equation (7), $Na_i' = 92$ mM; i.e., $K_i - K_i' = Na_i' - Na_i = 57$ mM. With these values for $\Delta\psi_{N-I}$, Na_i', and K_i' equation (9) then yields $\rho' = 0.049$. (The approximate equation (11) gives $\rho' = 0.046$.) ρ', it should be pointed out, is a characteristic of the whole innervated membrane; the contributions of the junctional and extrajunctional regions to ρ and ρ' will depend on the relative permeabilities of the two areas at rest and during a depolarization.

The results of the analysis are that in the first 75 seconds of an approximately half-maximal response 40 per cent of the intracellular K^+ exchanges for Na^+ and that the ratio of the permeability of Na^+ to that of K^+ increases fivefold. While the latter estimate seems quite reasonable, the former, although qualitatively consistent with the effect of ouabain on the repolarization, may be somewhat high. The net outward K^+ flux required for such an exchange is 5- to 50-fold greater than a value for the unidirectional K^+ flux previously reported.[6] The estimates of K_i and K_i', and hence of the flux, are subject to the errors inherent in the measurement of the resting potential with internal glass microelectrodes. The estimate of ρ', however, which depends most strongly on the transcellular potential, should be more reliable, and should provide, despite the simplifications involved, a better measure than the extent of depolarization of the primary response of the electroplax to acetylcholine and its congeners.

Summary.—Carbamylcholine-induced depolarizations of the isolated electroplax appear to involve initial changes in ion permeability and subsequent changes in intracellular ion concentrations. The significance of the concentration changes is indicated by the inhibition by ouabain of the repolarization and by the depolarization of the noninnervated and inexcitable membrane, as well as the innervated membrane, when carbamylcholine is applied to the innervated side. The changes in concentration and in permeability are estimated by an analysis based on the Goldman equation and on the functional asymmetry of the electroplax. The estimate of the permeability change is proposed as a measure of the primary response of the electroplax to acetylcholine and its congeners.

I thank Dr. D. Nachmansohn for his support and encouragement, Dr. I. Silman for his critical reading of the manuscript, and Miss E. C. Cunningham for her technical help.

* Supported by grants NB-07065 and NB-03304 from USPHS.

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