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ABOUT THE CHANGES OF INTERNAL IONIC CONCENTRATION IN THE ISOLATED ELECTROPLAX DURING CHEMICAL EXCITATION

ROBERT BLUMENTHAL AND JEAN-PIERRE CHANGEUX
Département de Biologie Moléculaire, Institut Pasteur, Paris (France)
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SUMMARY

Changes in the electrical potential established across the non-innervated membrane of the isolated electroplax occur when the innervated membrane is depolarized by bath application of either cholinergic agonists or a physiological solution containing a high concentration of K^+. Such changes do not therefore represent a decrease in internal K^+ concentration. That the intracellular concentrations of K^+ and Na^+ do not vary appreciably upon bath application of cholinergic agonists is further substantiated by measurements of the volume of the cell and of its total content of both potassium and sodium. This result is of importance for the interpretation of the steady changes of electrical potential caused on the innervated membrane by the cholinergic agonists.

INTRODUCTION

The isolated electroplax from the electric organ of *Electrophorus electricus* is one of the most convenient preparations for the study of the molecular mechanisms of membrane excitation[1]. On considering the importance of the conclusions which have been inferred from data obtained with this cell, it appeared to us to be of interest to reconsider in detail some of the basic features of the cell response to cholinergic agonists. One such feature is the variation in the ionic concentration of Na^+ and K^+ within the cell during bath application of cholinergic agonists. As already discussed by several authors, the electroplax is an asymmetrical cell and only one of its faces, the innervated membrane, is excitable. The function of the non-innervated membrane is to maintain an ionic gradient between the inside and the outside of the cell by means of active transport. Since ions flow along their electrochemical gradient during excitation, it is important to know to what extent the active transport mechanism can compensate for the depletion of ions caused by excitation. KARLIN[2] has considered this problem and concluded, on the basis of several experiments, that during 1–10 min of standard exposure to cholinergic agonists: (1) the intracellular K^+ concentration $[K^+]_i$ decreases 2-fold and (2) the intracellular Na^+ concentration $[Na^+]_i$, increases 10-fold. The experimental evidence presented in favor of these conclusions was as follows: (1) When the innervated membrane is depolarized by exposure to cholinergic agonists, the non-innervated membrane depolarizes as well, although to a lesser extent. (2) When ouabain, a specific inhibitor of active transport, is applied

to the non-innervated face, the repolarization of the innervated membrane, after exposure to cholinergic agonists, is inhibited.

The results presented in this paper indicate that none of these observations, in fact, are demonstrative of a change in internal ionic concentration. Direct measurements of ionic concentrations inside the cell show that during excitation the total K^+ or Na^+ concentrations do not change by more than 10%. The measurement of the steady-state potential, as used originally by HIGMAN *et al.*[1], thus constitutes a reliable index of the changes in properties of the excitable membrane.

MATERIALS

Electroplax from the organ of Sachs of *Electrophorus electricus* were dissected according to the improved method developed by SCHOFFENIELS[3]. Only undamaged electroplax with a fairly complete separation of the innervated membrane from the connective tissue of the contiguous compartment were employed.

The adherent water of the cell was drained against the smooth lip of a cylindrical beaker, by going six times progressively around the circumference of the beaker so that all sides of the preparation were drained, then the cell was weighed on a torsion balance (Mettler and Co.) (blotting on filter paper causes damage). The weights ranged between 4 and 35 mg. The analyses were carried out in the eel's Ringer solution[4] to which the various compounds were added.

All the radioactive compounds were from C.E.N., Radioisotopes Department (Mol, Belgium). Ouabain and carbamylcholine were from K and K laboratories.

METHODS

Chemical analyses

Electrolytes were extracted by soaking the cell in dilute acetic acid overnight. Identical results were obtained by soaking the cell in either 6 M HCl or 1% Triton X-100. $[Na^+]$ and $[K^+]$ were determined with a Zeiss spectrophotometer PMQ II provided with a flame attachment FA. Gas supplied to the burner was a mixture of O_2 and H_2. Absorbances were read at 766.5 nm for K^+, with a window width of 1.0 mm. A solution of $5 \cdot 10^{-4}$ M K^+ gives, in these conditions, an absorbance of 55.0. $[Na^+]$ was measured at 589 nm, with a window width of 0.008 mm. A solution of $5 \cdot 10^{-4}$ M Na^+ gives, in these conditions, an absorbance of 33.0.

Membrane potentials

Membrane potentials were determined according to the procedure of HIGMAN *et al.*[1]. The composition of Ringer's physiological solution is given in ref. 4. When $[K^+]$ was changed, $[Na^+]$ was changed as well, in such a manner that the total concentration of $Na^+ + K^+$ remained constant.

RESULTS

Effect of the variation of external potassium concentration on the electrical potential of the innervated and non-innervated membranes

According to KARLIN[2], variations of the electrical potential across the non-innervated membrane reflects a change in $[K^+]_i$. In order to test the validity of this inter-

pretation we decided to mimic the depolarization by cholinergic agonists at the innervated membrane by changing the external $[K^+]_0$ concentration in Ringer's fluid, K^+ being replaced by Na^+.

We first verified, as illustrated in Fig. 1, and in agreement with the result of HIGMAN *et al.*[1], that when K_0 varies the innervated membrane behaves like a potassium electrode: the slope of the Nernst plot of the data is 58 mV for a 10-fold increase in $[K^+]_0$ and the membrane potential becomes zero for $[K^+]_0 = 200$ mM. We then measured the membrane potential of the non-innervated membrane during depolarization of the innervated membrane by bath application of high $[K^+]_0$.

The driving force for inward K^+ movement is given by $V - V_K$, where V is the membrane potential and $V_K = RT \ln [K^+]_i/[K^+]_0$, the equilibrium potential for K^+. When $[K^+]_0$ increases V_K decreases, thus the driving force for inward K^+ movement should increase. If changes in the potential across the non-innervated membrane indeed represent changes in $[K^+]_i$, then an increase in $[K^+]_0$ should either give an increase of potential or no change at all. In any case, the potential should never decrease. In fact, as shown in Fig. 2, when the innervated membrane is depolarized by high $[K^+]_0$ the potential across the non-innervated membrane decreases as well. Changes of potential at this level do not thus represent changes in $[K^+]_i$. We therefore abandoned measurements of the electrical potential across the non-innervated membrane.

Effects of the variation of external potassium concentration on electrical potential after prolonged exposure to a cholinergic agonist, carbamycholine, and (or) to an inhibitor of active transport, ouabain

Another indication in favor of a change of $[K^+]_i$ following excitation might come from the use of inhibitors of active transport such as ouabain. Ouabain prevents the compensatory effect of active transport and thus should make the loss of ion due to excitation (or leak) more evident. It might even lead to a change in the equilibrium potential value for that ion (V_K).

First of all, Fig. 3 shows that the potential response to $6.5 \cdot 10^{-2}$ M $[K^+]_0$ measured across the innervated membrane after exposure of the non-innervated membrane to 10^{-4} M ouabain for 10 min is reversible and has the same amplitude as in control

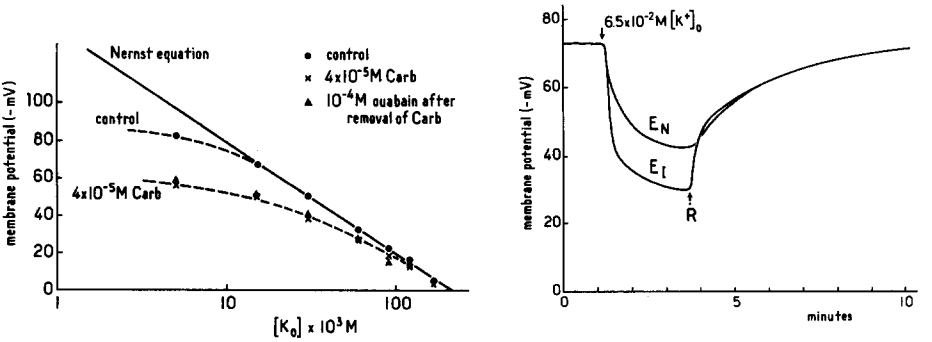


Fig. 1. Nernst plot of the variation of the membrane potential with $[K^+]_0$. See text. Carb, carbamylcholine.

Fig. 2. Depolarization of the innervated (E_I) and non-innervated (E_N) membrane by application of $6.5 \cdot 10^{-2}$ M $[K^+]_0$ to the innervated side. R, indicates application of Ringer's solution.

cells. In contrast, when $4 \cdot 10^{-4}$ M carbamylcholine is applied, the cell depolarizes as in the controls but the repolarization is, in agreement with KARLIN's result[2], strongly inhibited. The question is, then, does such an inhibition indeed represent a change in $[K^+]_i$? Fig. 3 shows that subsequent application of $6.5 \cdot 10^{-2}$ and $1.5 \cdot 10^{-1}$ M $[K^+]_0$ on

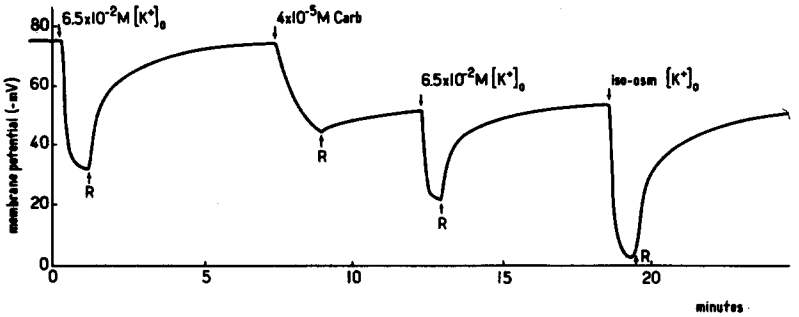


Fig. 3. Response of the innervated membrane to $[K^+]_0$ and carbamylcholine after exposure of 10^{-4} M ouabain to the non-innervated membrane for 10 min. The concentrations of $[K^+]_0$ and carbamylcholine (Carb) are indicated and R, indicates Ringer's solution.

the innervated membrane leads to almost the same absolute value of the membrane potential as in the control cells. Furthermore, the Nernst plot of a more complete set of data obtained when $[K^+]_0$ is varied from $5 \cdot 10^{-3}$ up to $1.6 \cdot 10^{-1}$ M in the presence of carbamylcholine, or in the presence of ouabain after exposure to carbamylcholine, extrapolate to almost the same value of $[K^+]_0$ at zero potential. If $[K^+]_i$ had changed by 50 % a noticeable shift in the position of the Nernst plot would have been detected. These results indicate that, within our experimental conditions, we have no indication for an appreciable change in $[K^+]_i$*.

Direct measurements of internal potassium concentrations by flame spectrophotometry

Measurement of extracellular space

Estimation of ionic concentrations within the cell requires, as a pre-requisite, determination of the volume of the cell. For this purpose we compared the wet weight of the cell with its external volume, estimated with radioactive [^{3}H]inulin (C.E.N. radioisotopes department, Mol, Belgium), a compound to which the cell is highly impermeable (see METHODS). Fig. 4 shows the kinetics of equilibration of a set of cells in the presence of inulin. Half-equilibration is achieved in about 15 min and after 2 h the equilibration was assumed to be complete. In these conditions, the extracellular space was 75 ± 5% of the total volume, assuming for the cell a density of 1. Other "impermeable" indicators, such as [*Me*-^{14}C]-β-D-thiogalactopyranoside or ^{22}Na, gave approximately the same results. Incidentally, our data are in agreement with those of DAVSON AND LAGE[6] who reported that, using ^{22}Na or chloride as indicators, and slices of electric organ, the extracellular space constitutes 70% of the total volume of the electroplax in the caudal region of the eel.

* Recently RUIZ-MANRESA[5] has measured current–voltage relationships of the innervated membrane of the electroplax after exposure to a maximal dose of carbamylcholine. Under these conditions V_K does not change.

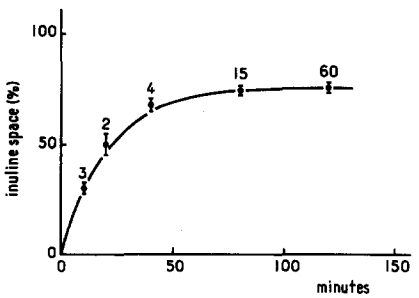
The invariance of internal volume upon depolarization by Carbamylcholine

We considered the possibility that in the course of excitation an appreciable change in the volume of the cell might occur, which would, as a consequence, contribute to a change in internal ionic concentration. We therefore measured the permeability of the cell to water before, during and after excitation. For this purpose we followed the passive transport of tritiated water across a single electroplax mounted according to the procedure of HIGMAN *et al.*[1]. Ringer's solution containing ^{3}HHO (0.5 mC/ml) was applied to the compartment facing the non-innervated membrane and normal Ringer's solution was passed at a rate of 8 ml/min through the compartment on the innervated side. The radioactivity of the solution bathing the innervated membrane rapidly reaches a steady state which corresponds to the permeability of the cell to water at rest. Then 10^{-4} M carbamylcholine was added to the washing solution, giving a maximal depolarization of the cell. Fig. 5 shows the result of such an experiment: a 61 mV depolarization of the cell, which causes a 4-fold transient increase in potassium efflux[1], does not produce a change in water permeability within 10% accuracy. The permeability to water (Pd) across the cell is $2.35\ (\pm\ 0.23)\cdot 10^{-4}$ cm/sec. This result may be compared with the results of VILLEGAS AND VILLEGAS[7], who obtained a Pd of $1.42 \pm (0.39)\cdot 10^{-4}$ cm/sec in the giant nerve fiber of *Doryteuthis plei*. Since no appreciable change of water permeability occurs upon excitation, we therefore conclude that no appreciable volume changes have to be taken into account.

Direct determination of total internal potassium and sodium concentrations

Since the volume of the cell does not change significantly upon excitation, direct measurement of the ionic content of the cell should give an estimate of the actual concentration of the ion under consideration in the cell. The total content of Na^+ and K^+ was measured by means of flame spectrophotometry after extraction of cations by dilute acetic acid (see METHODS). In Table I are shown the total contents of K^+ and Na^+ in cells exposed for various lengths of time to 10^{-4} M carbamylcholine, 10^{-4} M ouabain and to both these compounds.

It is clear that a depolarization of 10 min, performed either in the presence



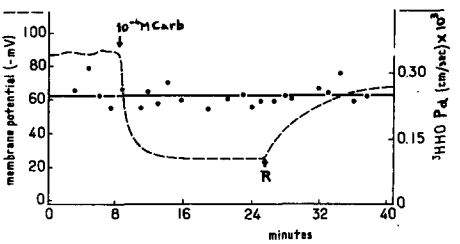


Fig. 4. Rate of equilibration of [^{14}C]inulin with electroplax at 23°. The dots represent mean values. Standard errors and the number of determinations for each mean value are indicated.

Fig. 5. Membrane potential and water permeability as a function of time after application of 10^{-4} M carbamylcholine (Carb) and Ringer (R) as indicated. The water permeability is defined as $Pd = J/\rho$, where J is the ^{3}HHO flux per unit area (counts/min in the superfluent)/(sec·area of the window) and ρ the specific activity of ^{3}HHO in the bathing solution.

TABLE I

$[K^+]_i$ AND $[Na^+]_i$ IN SINGLE ELECTROPLAX CELLS

The concentrations are in mM/kg cells after correction for extracellular space.

Medium + additions (10^{-4} M)	*Time of application (min)*	$[K^+]_i$	$[Na^+]_i$	*Number of cells*
Ringer's solution		160.1 ± 8.9	11.8 ± 6	67
+ carbamylcholine	1	159.5 ± 9.0	14.5 ± 24.6	15
+ carbamylcholine	10	153.9 ± 7.1	7.9 ± 30.7	23
+ carbamylcholine	20	152.2 ± 4.7	18.1 ± 18.4	11
+ carbamylcholine + Ringer's solution	10 + 10	158.9 ± 8.8	14.8 ± 13.5	9
+ ouabain	10	155.5 ± 7.5	16.6 ± 26.2	12
+ ouabain	180	131.3 ± 11.7	37.3 ± 35.0	11
+ ouabain + ouabain + carbamylcholine	10	152.2 ± 9.6	16.7 ± 17.1	9
+ ouabain + carbamylcholine	180	122.1 ± 5.8	35.7 ± 30.3	11

or in the absence of ouabain, does not produce any significant change (less than 10%) in $[Na^+]$ and $[K^+]$. Only prolonged exposure of several hours to ouabain in the presence or the absence of carbamylcholine gives an appreciable change. Excitation of the cell, following a standard procedure of a few minutes, therefore, does not produce any significant change in the total $[K^+]$ or $[Na^+]$ in the isolated electroplax.

DISCUSSION

Using both electrophysiological and chemical methods, we are unable to demonstrate any significant change in the total concentration of ions in the electroplax, during chemical excitation. The changes in electrical potential at the non-innervated membrane, therefore, cannot be considered to provide an index of internal ionic concentration; such changes may rather represent some kind of electric coupling between the innervated membrane and the non-innervated membrane, or simply changes in electrical potential at the innervated membrane outside the window surface. The effect of ouabain is rather difficult to interpret. Either it represents local changes in the ionic gradients which are small enough to leave the total ionic concentration unchanged (this implies that some kind of compartmentalization exists within the cell), or ouabain applied at the non-innervated membrane crosses the cell and becomes an effector of the inner face of the innervated membrane through some kind of pharmacological action distinct from inhibition of active transport. In any case, although ions flow passively across the innervated membrane during excitation, the volume of the cell is large enough and the active transport sufficiently high, that no appreciable alteration of the steady concentration of ions within the cell is detectable. This result is of importance for the quantitative interpretation of the cell response. If appreciable changes in ionic concentration were involved in the course of this response, the actual measurement of the steady-state potential at a given concentration of agonist would not strictly represent changes in properties of the innervated membrane. The present

results support the conclusions of HIGMAN *et al.*[1] and of CHANGEUX AND PODLESKI[8] that these steady-state potentials are reasonable indices of the permeability changes occurring at the innervated membrane. This conclusion is further supported by the observation of KASAI AND CHANGEUX[9] that the dose–response curves obtained by measuring the steady-state potentials, *in vivo*, and Na^+ fluxes across purified membrane bags, *in vitro*, superimpose almost exactly.

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REFERENCES

1 H. B. HIGMAN, T. R. PODLESKI AND E. BARTELS, *Biochim. Biophys. Acta*, 75 (1963) 187.
2 A. KARLIN, *Proc. Natl. Acad. Sci. U.S.*, 58 (1967) 1162.
3 E. SCHOFFENIELS, *Biochim. Biophys. Acta*, 26 (1957) 585.
4 R. WHITTAM AND M. GUINNEBAULT, *Biochim. Biophys. Acta*, 45 (1960) 336.
5 F. RUIZ-MANRESA, *Ph. D. Thesis*, Columbia University, 1970.
6 H. DAVSON AND H. V. LAGE, *An. Acad. Bras. Ciências*, 25 (1953) 303.
7 R. VILLEGAS AND G. M. VILLEGAS, *J. Gen. Physiol.*, 43 (1960) 73.
8 J. P. CHANGEUX AND T. R. PODLESKI, *Proc. Natl. Acad. Sci. U.S.*, 59 (1968) 944.
9 M. KASAI AND J. P. CHANGEUX, *Compt. Rend. Acad. Sci. Paris*, 270 (1970) 1400.