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# Surface Charges on the Outer Side of Mollusc Neuron Membrane

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**Summary.** The shifts of current-voltage characteristics of sodium and calcium inward currents produced by changes in the concentration of divalent cations ($Mg^{2+}$, $Ca^{2+}$, $Sr^{2+}$, $Ba^{2+}$) and in pH of the extracellular solution have been measured on isolated neurons of the mollusc *Helix pomatia* intracellularly perfused with potassium-free solutions. On the basis of these shifts and using Stern's theory (O. Stern, 1924, *Z. Electrochem.* **30**:508–516), the binding constants for the ions to charged groups of the outer side of the somatic membrane and the density of the surface charges produced by these groups have been calculated. For groups located in the vicinity of sodium channels we obtained $K_{Ca}=90\pm10$, $K_{Sr}=60\pm10$, $K_{Ba}=25\pm5$ and $K_{Mg}=16\pm5\,M^{-1}$ at pH=7.7 and for groups located in the vicinity of calcium channels $K_{Ca}=67\pm10$, $K_{Sr}=20\pm5$ and $K_{Ba}=18\pm5\,M^{-1}$ at pH=7.0. The same groups bind $H^+$ ions with apparent pK=6.2±0.2 that corresponds to $K_H=1.6\times10^6\,M^{-1}$. The density of fixed charges near the sodium channels is $0.17\pm0.05$ e/nm$^2$ (pH=7.7) and near the calcium channels is $0.23\pm0.05$ electrons/nm$^2$ (pH=7.0). From the comparison of the obtained values with the data about binding constants of the same ions to different negatively charged phospholipids, a suggestion is made that just the phosphatidylserine is responsible for the surface potential of the outer side of the somatic membrane. It was also shown that the presence of this potential results in a change in the concentration of carrier ions near the membrane which affects the maximal values of the corresponding transmembrane currents.

**Key Words** surface potential · Stern's theory · mollusc neurons

## Introduction

The giant neurons of molluscs have been widely used during recent years for the investigation of the ionic mechanisms of electric excitability of the somatic membrane. A much more complicated system of electrically operated ionic channels has been found in this membrane compared with the axonal one. A quantitative description of this system needs exact data about the events occurring on the surface of the somatic membrane. A well-known example of the effect of such events is the shift of the potential-dependent characteristics of the ionic currents along the potential axis depending upon the composition of the surrounding solution. This effect has been observed on the membrane of squid giant axon (Chandler, Hodgkin & Meves, 1965), on the Ranvier node membrane of frog myelinated fibers (Mozhayeva & Naumov, 1972*a*, *b*, *c*; Hille, Woodhull & Shapiro, 1975), on the membrane of oocytes (Ohmori & Yoshii, 1977) and mollusc neurons (Kostyuk & Krishtal, 1977). Usually this shift is attributed to the effect of surface potential of the membrane and for a quantitative description of this effect the authors have used the electric double-layer models developed in electrochemistry first by Gouy (1910) and Chapman (1913) and later by Stern (1924). On the basis of these models the data about the density of charged membrane groups and their binding constants for different ions have been obtained (Gilbert & Ehrenstein, 1970; McLaughlin, Szabo & Eisenman, 1971; Mozhayeva & Naumov, 1972*a*, *b*, *c*; Begenisich, 1975).

In the present work we determined the effect of $H^+$ and alkali-earth ($Mg^{2+}$, $Ca^{2+}$, $Sr^{2+}$, $Ba^{2+}$) ions in the extracellular solution on the current-voltage characteristics of inward currents in the mollusc somatic membrane. Using Stern's model, the binding constants of these ions to charged groups of the outer side of the membrane and their density have been calculated. On the basis of the obtained data the suggestion has been made about the chemical nature of these groups.

A preliminary communication on the work has been published elsewhere (Kostyuk, Doroshenko & Ponomarev, 1979).

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## Theory

According to the double electric layer theory, the introduction of ions into the solution bathing the membrane surface leads to changes of its potential by both partial neutralizing surface charged groups and by forming a diffuse layer of counter-ions. The relation between the density of charges at the membrane surface $\sigma$ and its potential $\varphi$ is given by the following expression (Chandler et al., 1965):

$$\frac{\varepsilon}{4\pi}\left(\frac{d\varphi}{dx}\right)_{\text{out}} - C(\varphi_{\text{out}}-\varphi_{\text{in}}) = -\sigma \tag{1}$$

where $\varepsilon$ is the dielectric constant of the solution, $\varphi_{\text{out}}(\varphi_{\text{in}})$ is the surface potential of the outer (inner) surface of the membrane, $C$ is the membrane capacity. Assuming that $C \simeq 1\,\mu\text{F/cm}^2$ (Magura, Grobova & Zamekhovsky, 1972) and $|\sigma| > 0.02\,\text{e/nm}^2$, the relative contribution of the second term to Eq. (1) at potentials lower than 50 mV in absolute value will not exceed 10% and at first approximation it can be neglected. In this case the relation between $\sigma$ and $\varphi = \varphi_{\text{out}}$ can be written in the Gouy-Chapman form:

$$\sigma = \pm\sqrt{\frac{\varepsilon RT}{2\pi}\Sigma C_i(\exp(-z_i\varphi F/RT)-1)} \tag{2}$$

where $C_i$ is volume concentration of the ion $i$ and $z_i$ is its charge.

Considering the effect of complex formation on the charge density of the membrane surface, let us, for simplicity, assume that these charges correspond to the functional groups of only one type ($X$) present at the membrane surface in the form of single charged anions ($X^-$). So far as the extracellular solution contains ions of different valency (at least mono- and divalent), the latter can form surface complexes of different types. Let us consider two extreme cases: a) all ions form complexes of 1:1 type; b) the ion of each type forms a complex, the stoichiometry of which corresponds to the valency of the ion.

In the first case the occupation number of surface complexes formed by the ion $i$ having the charge $z_i$ is:

$$\theta_i = K_{M_i}[M^{z_i}](1-\theta) \tag{3}$$

where $\theta = \Sigma\theta_i$, $[M^{z_i}]$ being the near-membrane concentration of the ion $i$ and $K_{M_i}$ is the equilibrium constant of the reaction

$$M^{z_i} + X^- \rightleftarrows MX^{(z_i-1)}. \tag{4}$$

Multiplying $\theta_i$ by the charge of the complex, which is equal to $(z_i-1)$, and taking into account that the amount of free groups is $1-\theta$, we find the value of charge density of the surface

$$\sigma = \sigma_o[(1-\theta)-\Sigma(z_i-1)\,\theta_i] = \sigma_o\frac{1-\Sigma z_i K_{M_i}[M^{z_i}]}{1+\Sigma K_{M_i}[M^{z_i}]} \tag{5}$$

where $\sigma_o$ is the surface charge density when all surface groups are free.

In the second case the formation of complexes can be described by the equation:

$$M^{z_i} + z_i X^- \rightleftarrows MX_{z_i}. \tag{6}$$

In this case all surface complexes are neutral; therefore the value of $\sigma$ is determined by the number of unbound groups, which is proportional to $(1-\theta)$. From the expression of binding constant, corresponding to Eq. (6), it can be shown that the value of $(1-\theta)$ can be obtained as a solution of polynomial equation, the order of which is equal to the highest valency of ion in the bathing solution. To avoid this difficulty we assume for simplicity that these surface groups are present in elementary units (pairs, triads, etc.) which bind the ions of corresponding valency. The occupation number of such units is equal to $(1-\theta)/z_i$ and for the occupation number of surface complexes we have the following expression:

$$\theta_i = K_{M_i}[M^{z_i}](1-\theta)/z_i. \tag{7}$$

Because the occupation number of surface groups in complexes of $MX_z$ type is $z_i$ times larger than $\theta_i$, we obtain that

$$\theta = \frac{\Sigma K_{M_i}[M^{z_i}]}{1+\Sigma K_{M_i}[M^{z_i}]}. \tag{8}$$

The expression for surface charge density now takes the form:

$$\sigma = \sigma_o(1-\theta) = \frac{\sigma_o}{1+\Sigma K_{M_i}[M^{z_i}]}. \tag{9}$$

Comparing expressions (5) and (9) we see that the values of $\sigma$ in the limits of large concentrations of ions ($C_i \gg 1/K_i$) are quite different:

$$\lim_{C_i\to\infty}\sigma = (1-z_i)\,\sigma_o \tag{10}$$

$$\lim_{C_i\to\infty}\sigma = 0. \tag{11}$$

Thus, depending on the type of interaction between multicharged ions and functional groups at the membrane surface, either its neutralization or overcharging may occur. Intermediate cases can also happen in the system considered. They are described by the following chain of reactions:

$$M^{z+} + X^- \rightleftarrows MX^{(z-1)+}$$
$$MX^{(z-1)+} + X^- \rightleftarrows MX^{(z-2)+} \ldots . \quad (12)$$

The actual mechanism of polyvalent ion interaction with biological membranes as well as with their possible prototypes - artificial phospholipid membranes - is not established at present. Most of the experimental data made on artificial membranes (Papahadjopoulos, 1968; McLaughlin et al., 1971; Traube & Eibl, 1974; Nir, Newton & Papahadjopoulos, 1978; Hammoudan et al., 1981; Ohki & Kurland, 1981) indicate that the complexes of $MX_z$ type are predominantly formed on the surfaces of the lipid membranes. However, McLaughlin et al. (1981) using a microelectrophoresis technique observed the reversal of charge for multilamellar phosphatidylserine (PS) vesicles upon the addition of different divalent ions in concentration of about 0.1 M. This effect can be hardly explained considering only the formation of stoichiometric complexes described by Eq. (6); but at such concentrations of divalent ions, where charge reversal of PS vesicles does occur, such phenomena as the formation of nonbilayer phases, aggregation of vesicles, etc., are involved (*see* Cullis & De Kruijff, 1979). In this case there might be another type of divalent ion binding with charged phospholipid molecules as compared with that occurring in bilayer membranes formed by these molecules. This is supported by the work of Hammoudan et al. (1981) where it was shown by differential scanning calorimetry that the PS phase disappears if the ratio of $Ca^{2+}$ ions bound to PS molecules equals 1:2. That is why in our calculations we suggested that the processes described by Eq. (6) are mainly responsible for the changes in the density of surface charges at the outer side of the somatic membrane of mollusc neurons.

We have also suggested that only protons and divalent ions (Ca, Sr, Ba, Mg) bind to the membrane surface but not the monovalent ones (e.g., $Na^+$, $K^+$, $Tris^+$). The latter assumption is based on the fact that the binding constants of monovalent ions to different anions are of 1–2 orders less than those of multivalent ions and protons (*see*, e.g., Martell & Smith, 1977, and Discussion).

With these assumptions Eq. (9) becomes

$$\sigma = \frac{\sigma_o}{1 + K_{M^{2+}}[M^{2+}] + K_{H^+}[H^+]}. \quad (13)$$

For small near-membrane concentrations $[M^{2+}]$ and $[H^+]$ compared with the value of $1/K_i$, the term $K_{M^{2+}}[M^{2+}]\,K_{H^+}[H^+]$ can be added to the denominator of Eq. (13) and then we have:

$$\sigma = \frac{\sigma_o}{(1 + K_{M^{2+}}[M^{2+}])(1 + K_H + [H^+])} = \frac{\sigma'_o}{1 + K_{M^{2+}}[M^{2+}]}. \quad (14)$$

The obtained expression can be interpreted in the following way: if ions $H^+$ and $M^{2+}$ are added to the bathing solution, the complexes $HX$ are formed first and then the free $X^-$ groups, the surface concentration of which is now $\sigma'_o/e$ interact with divalent cations and form complexes $MX_2$.

Substituting in Eq. (14) the near-membrane concentration for volume concentration

$$[M^{z_i}] = C_i \exp(-z_i \varphi F/RT) \quad (15)$$

and equating the right parts of Eqs. (2) and (14), we obtain the following relation:

$$\frac{|\sigma'_o|}{1 + K_{M_i} C_i \exp(-z_i \varphi F/RT)} = \sqrt{\frac{\varepsilon RT}{2\pi} \Sigma C_i (\exp(-z_i \varphi F/RT) - 1)} \quad (16)$$

which has been used for all calculations.

## Materials and Methods

Experiments were made on unidentified isolated dialyzed neurons of the snail *Helix pomatia*. The techniques of cell isolation and intracellular perfusion have been described earlier (Kostyuk, Krishtal & Doroshenko, 1974; Kostyuk, Krishtal & Pidoplichko, 1975, 1981). In the course of perfusion the intracellular ionic content has been substituted for Tris-glutamate (170 mM) and EDTA (10 mM) at pH = 7.3 that enabled a complete elimination of the fast and delayed potassium outward currents. In this way an undistorted recording of the inward sodium and calcium currents has been achieved within the testing potential range up to +50 mV. At more positive testing potentials the recordings were less precise because of the superposition of a nonspecific outward current carried by $Tris^+$ ions (Kostyuk & Krishtal, 1977). For a separate recording of sodium inward currents fluoride anions have been in-

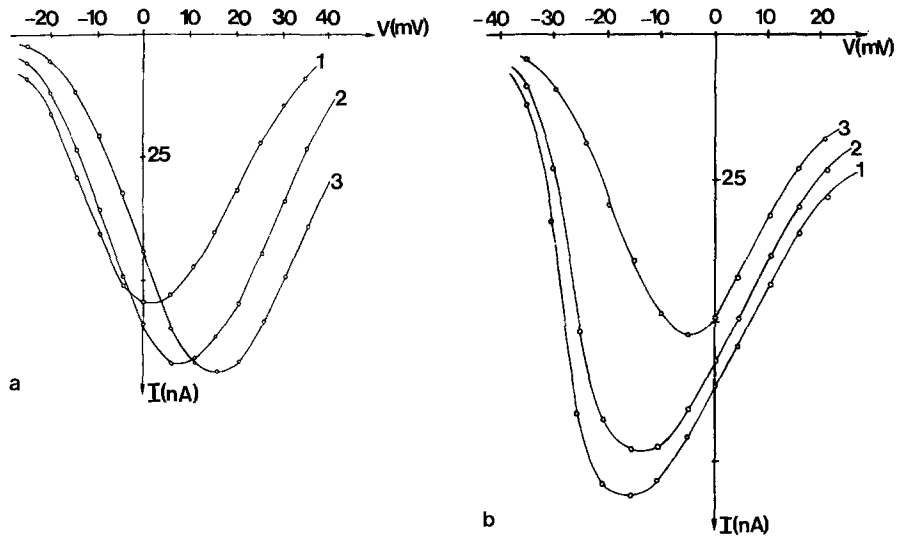


**Fig. 1.** Typical current-voltage characteristics for calcium (*a*) and sodium (*b*) inward currents obtained for different extracellular concentrations of $Ca^{2+}$ ions. Curves were drawn through experimental points by eye. *a*) 1–10 mM: 2–30 mM; 3–60 mM $Ca^{2+}$; *b*) 1–2 mM; 2–5 mM; 3–60 mM $Ca^{2+}$

**Table 1.** Composition of extracellular solutions (all concentrations are in mM)

| | $MCl_2$[a] | NaCl | Tris-Cl | Sucrose |
|---|---|---|---|---|
| Sodium currents | 2 | 150 | 20 | 70 |
| | 5 | 150 | 20 | 70 |
| | 10 | 150 | 20 | 70 |
| | 60 | 150 | 20 | 70 |
| Calcium currents | 10 | – | 170 | 10 |
| | 30 | – | 110 | 60 |
| | 60 | – | 20 | 150 |

[a] $M-Ca^{2+}$; $Ba^{2+}$ or $Sr^{2+}$ ions.

troduced into the cell which selectively destroy the calcium conductance in the somatic membrane (Kostyuk et al., 1975). A separate recording of calcium inward currents was made after replacement of $Na^+$ ions in the extracellular solution by $Tris^+$, the cell being perfused with glutamate. To affect the surface potential of the membrane, the following testing extracellular solutions have been used (Table 1). The pH of the extracellular solutions has been adjusted by buffers with Tris-OH and Tris-glutamate and controlled throughout the experiment. In the course of long experiments on isolated cells a slow decline in the maximal amplitude of ionic currents always happened due to a decrease in the number of functioning ionic channels in the membrane. Therefore, repeated measurements of ionic current in the starting extracellular solution were made on each cell, and the results of intermediate measurements were normalized by linear interpolation.

The changes in the surface potential have been evaluated from the shifts in the falling branch of the corresponding current-voltage curve along the potential axis. In this potential range the amplitude of the current is determined mainly by the process of activation of ionic channels and can be described by the Hodgkin-Huxley equation using the cubic power of the *m*-variable for the sodium currents and the square power for the calcium currents (Kostyuk & Krishtal, 1977). The potential-dependence of $m_\infty$ has been approximated as follows:

$$m_\infty = (I/I_{max})^{1/a} = [1 + \exp(-b(V - V_{\frac{1}{2}}))]^{-1} \quad (17)$$

where $a=3$ in the case of sodium and $a=2$ in the case of calcium currents, $I_{max}$ corresponds to the maximum of current-voltage characteristics and $I$ is the steady-state current at testing potential $V$, $b$ is the steepness parameter, and $V_{\frac{1}{2}}$ is the testing potential at which the half-level of activation is achieved.

For each type of current the mean value of the parameter $b$ has been found for 7–10 cells tested and then the mean $\bar{V}_{\frac{1}{2}}$ values were determined for different concentrations of $H^+$ and $M^{2+}$ ions (calculations made using at least 20 current-voltage curves).

## Results

Figure 1 presents typical current-voltage characteristics of sodium and calcium currents obtained in solutions with different concentration of $Ca^{2+}$ ions. Figure 2 shows similar characteristics for inward currents when the external solution contains different divalent cations at the same concentration. Both Figures show the shift of the falling branch of the current-voltage characteristics corresponding to the voltage-dependent process of ionic channel activation along the potential axis. We interprete this effect as the change of the surface potential of the outer side of the cell membrane (*see also* Chandler et al, 1965; Gilbert & Ehrenstein, 1970).

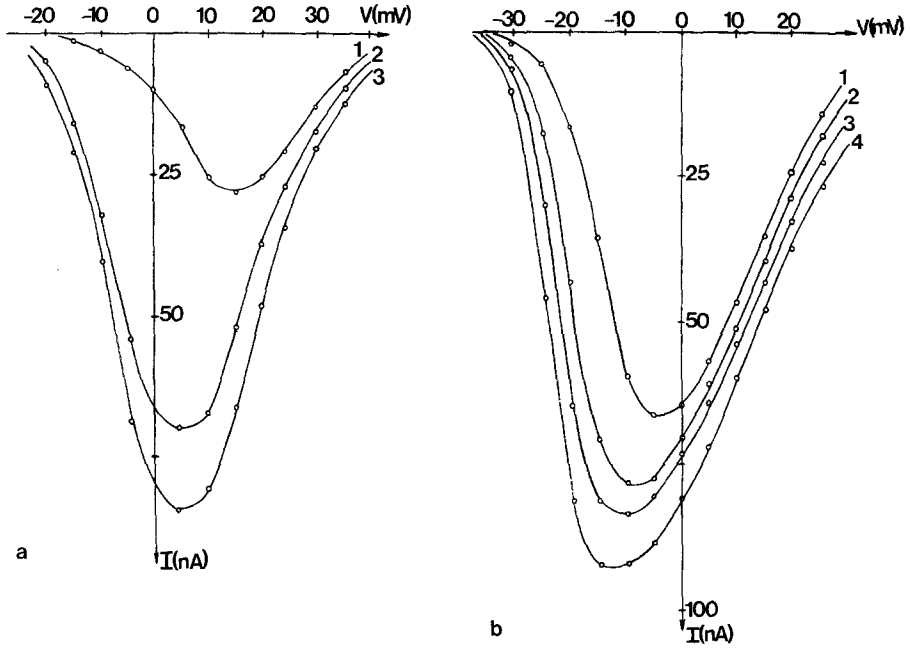


**Fig. 2.** Typical current-voltage characteristics for calcium (*a*) and sodium (*b*) inward currents obtained for different types of divalent cations ($1-Ca^{2+}$, $2-Sr^{2+}$, $3-Ba^{2+}$, $4-Mg^{2+}$) in extracellular solution. *a*) $C_{M^{2+}}=10$ mM; *b*) $C_{M^{2+}}=5$ mM. Curves were drawn through experimental points by eye

In parallel with this shift, a regular increase of the maximal value of the sodium current can be seen with a decrease in the extracellular concentration of $Ca^{2+}$ ions (Fig. 1b), as well as with the substitution of other divalent cations for calcium (Fig. 2*b*). This effect can also be attributed to the change in the value of surface potential leading to a corresponding change of the concentration of carrier ions near the membrane surface (*see Discussion*). For the calcium current, however, this effect could not be revealed, because in this case the divalent ions are themselves the charge carriers.

Equation (16), used for the analysis of the obtained data, includes 3 unknown variables: $\sigma_o'$, $K_M$ and $\varphi$. The value of $\varphi$ can be considered as the parameter of the model, because in our experiment we measure not the value of surface potential but its relative change, which depends on the composition of the bathing solution. Thus, it is convenient to define $\varphi$ as follows:

$$\varphi = -\bar{V}^o_{\frac{1}{2}} + \bar{V}_{\frac{1}{2}} \tag{18}$$

where $\bar{V}_{\frac{1}{2}}$ is obtained from experimental data using Eq. (17), and $\bar{V}^o_{\frac{1}{2}}$ we define as $\bar{V}^o_{\frac{1}{2}} = \bar{V}_{\frac{1}{2}}$ in the absence of fixed charges on the membrane surface. Using the experimentally obtained values of $\bar{V}_{\frac{1}{2}}$ for three different extracellular concentrations of $Ca^{2+}$ (10, 30 and 60 mM) and combining Eqs. (16) and (18), the values of $\sigma_o'$, $K_{Ca}$ and $V^o_{\frac{1}{2}}$ have been found by the method of successive approximations. Thus, having known the value of $V^o_{\frac{1}{2}}$ we were able to determine the values of surface potential $\varphi$ for any composition of extracellular solutions used in our experiments, and to calculate $K_M$ values for $Sr^{2+}$, $Ba^{2+}$ and $Mg^{2+}$ ions according to Eq. (16). Figure 3 demonstrates that the magnitude of the shift of the falling branch of the corresponding current-voltage characteristic can be successfully approximated in the framework of our version of Stern's theory using the calculated values of model parameters $\sigma_o'$ and $K_M$.

The values of $\sigma_o'$ and $K_M$ obtained for sodium current at pH = 7.7 and for calcium current at pH = 7 are listed in Table 2. The $\sigma_o'$ values for other pH values were calculated in the same manner and for the calcium and sodium currents can be approximated by Langmuir's isotherm:

$$\sigma_o' = \frac{\sigma_o}{1+K_{H^+}[H^+]} = \frac{\sigma_o}{1+10^{\log K+\log[H^+]}} = \frac{\sigma_o}{1+10^{pK-pH}} \tag{19}$$

with $\sigma_o = 0.24 \pm 0.02$ e/nm$^2$ and $pK = 6.2 \pm 0.1$ which corresponds to $K_H = (1.6 \pm 0.4) \times 10^6$ M$^{-1}$ (*see* Fig. 4).

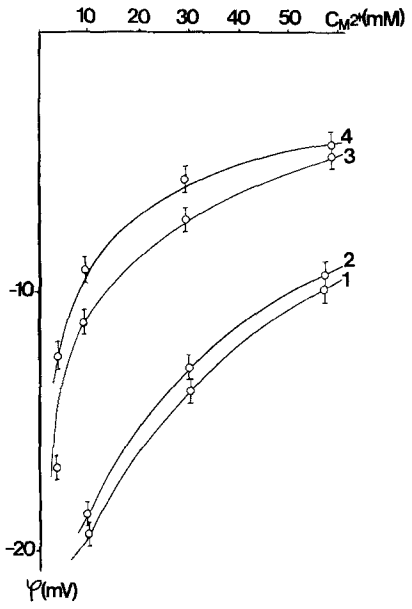


**Fig. 3.** Dependence of the value of surface potential $\varphi$ on $C_{M^{2+}}$. Curves were calculated according to Eq. (16); points correspond to experimental data. Vertical bars indicate experimental errors. 1, 2, 3 - calcium channel ($M$ = Sr, Ba, Ca); 4 - sodium channel ($M$ = Ca)

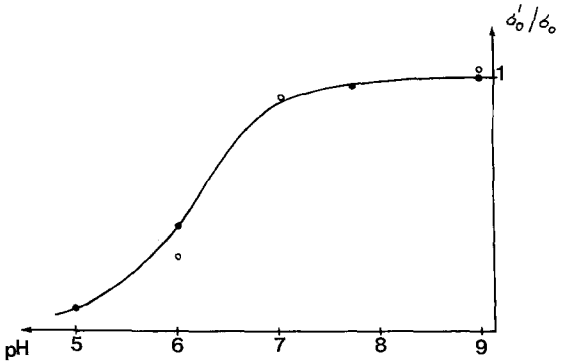


**Fig. 4.** Dependence of $\sigma_o'/\sigma_o$ on pH. ●, sodium channel; ○, calcium channel. Smooth curve was drawn according to Eq. (19)

## Discussion

The obtained data about the characteristics of charged groups on the outer side of the somatic membrane can be compared with similar data obtained on the other membranes. The mean distance between surface charges located in the vicinity of calcium and sodium channels, according to our calculations, is 20–24 Å. This value is close to the value obtained by Mozhayeva and Naumov (1972*a*, *b*, *c*) for frog Ranvier node, but is larger than those reported for other neuronal membrane surfaces, e.g., for sodium channels it was 11 Å for *Myxicola* axonal membrane (Begenisich, 1975), 10 Å for frog Ranvier node (Hille et al., 1975), and 9 Å for oocytes membrane (Ohmori & Yoshii, 1977).[1]

Our data about the values of $K_{Ca}$ also differ from the value of $K_{Ca} < 0.1\,\text{M}^{-1}$ (Begenisich, 1975), $K_{Ca} \simeq 0.1\,\text{M}^{-1}$ (McLaughlin et al., 1971), $K_{Ca} = 0.2\text{–}0.5\,\text{M}^{-1}$ (Ohmori & Yoshii, 1977), $K_{Ca} = 5\,\text{M}^{-1}$ (Mozhayeva & Naumov, 1972*c*). To our opinion, this large discrepancy is due to the different procedures employed for the determination of $K_{Ca}$ value. We obtained all unknown variables ($\sigma_o'$, $K_M$, $V_{\frac{1}{2}}^o$) simultaneously, while in the above-mentioned works the following procedure was adapted: using the experimental shifts of potential-dependent characteristics of ionic channel, the values of $\sigma_o'$ were obtained using the Gouy-Chapman theory and then, if necessary, the calculated dependence of surface potential was corrected taking into account the process of binding of these ions to surface groups.

For the values of $|\varphi| < RT/F$, we can expand the exponent in the square root of Eq. (16) in powers of $\varphi$. Then, neglecting the terms of the

[1] Here we discuss only cases in which Stern's theory was used, i.e., both $K_M$ and $\sigma_0'$ values have been determined.

**Table 2.** Values of $K_M(\text{M}^{-1})$ and $\sigma''_0(\text{e/nm}^2)$ obtained for functional groups located near inward current channels of the somatic membrane of mollusc neurons

| | pH | $K_{Ca}$ | $K_{Sr}$ | $K_{Ba}$ | $K_{Mg}$ | $\sigma_0'$ |
|---|---|---|---|---|---|---|
| Calcium channel | 7.0 | 67±10 | 20±5 | 18±5 | – | 0.23±0.05 |
| Sodium channel | 7.7 | 90±10 | 60±10 | 25±5 | 16±5 | 0.17±0.05 |

third and the higher powers of $\varphi$, we obtain from Eq. (16) the following expression:

$$\sigma = \frac{\sigma'_o}{1 + K_{Ca} C_{Ca} \exp(-2\varphi F/RT)} = \sqrt{\frac{\varepsilon RT}{2\pi} J} \cdot \varphi \tag{20}$$

where $J$ is the ionic strength of the solution. When the concentration of monovalent (divalent) ions is equal to $C_1(C_2)$, then $J = 3C_2 + C_1$. Thus, from Eq. (20) for $C_2 > C_1$ we have $\varphi \sim \sigma/\sqrt{C_2}$. Hence, we see that the decrease of $|\varphi|$ on the increase of $C_{Ca}$ is connected with both the decrease of surface charge density $|\sigma|$ due to binding of $Ca^{2+}$ ions to surface groups and the screening of surface potential by $Ca^{2+}$ ions (increase of ionic strength of the solution leads to the compression of double electric layer). So, it is evident that Gouy-Chapman and Stern's theories give the same value of surface potential shift only then, when the value of $|\sigma|$ in the former is larger than in the latter. It also follows from Eq. (20) that the larger values of $|\sigma|$ correspond to more negative values of $\varphi$.

It is important to note that the value of $K_{Ca}$ enters the basic Eq. (16) in the form of $K_{Ca} C_{Ca} e^{-2\varphi F/RT}$; therefore we should compare the values of $K_{Ca} e^{-2\varphi F/RT}$ rather than $K_{Ca}$. If we take into account that our values of $\varphi$ are 30–40 mV less negative than reported by above-mentioned authors, we find that the values of $K_{Ca} e^{-2\varphi F/RT}$ in these works and ours do not differ too much.

From these considerations it follows that the experimentally observed shifts of potential-dependent characteristics of ionic channels can be approximated using Stern's theory either with large $\sigma'_o$ and small $K_{Ca}$ or small $\sigma'_o$ and large $K_{Ca}$. Because we do not know the absolute value of surface potential we cannot distinguish between these two cases. However, experimental determination of $Ca^{2+}$ and $Mg^{2+}$ binding constants to the artificial phospholipid membranes along with the measurement of their $\zeta$-potential (Nir, Newton & Papahadjopoulos, 1978; Hammoudan et al., 1981; McLaughlin et al., 1981; Ohki & Kurland, 1981) support our results (*see below*).

It is known that phosphatidylserine (PS) is the main negatively charged phospholipid of biological membranes (its usual content is approximately 15%) (Cullis & de Kruijff, 1979). If we take into account that phospholipids occupy an area of 70 Å$^2$ in the membrane (Papahadjopoulos, 1968; Traube & Eibl, 1974; McLaughlin et al., 1981), the average charge density produced by PS in biological membrane must be equal to 0.21 e/nm$^2$, which is close to the values calculated in the present work (Table 2).

Eisenberg, Gresalfi, Riccio and McLaughlin (1979) have shown that monovalent cations can bind to membranes containing negative phospholipids, their binding constants being 10–100 times less than those for divalent cations. If this binding is not taken into account, one can obtain from experimental data only effective charge density and apparent binding constants for divalent cations. These values differ from the corresponding intrinsic values by a factor $(1 + K_M C_M \exp(-\varphi F/RT))$ where $M$ stands for monovalent cation used in experiments. Using $K_M = 0.6\,\text{M}^{-1}$ obtained by Eisenberg et al. (1979) for sodium ions, $C_M = 0.15\,\text{M}$ (Table 1) and $\varphi = -25\,\text{mV}$ (Fig. 3), we obtain the maximal error due to our neglecting the monovalent ion binding to the membrane less than 20%. We think that this value is within the range of our experimental error (*see* Table 2). The difference between the values of $\sigma'_o$ obtained for sodium and calcium channels (*see* Table 2) seems to be due to the fact that the measurements of calcium currents were performed in the presence of $Tris^+$ ions which have lesser affinity to negative phospholipids than sodium ions (*see* McLaughlin et al., 1981) used in our studies of characteristics of surface negative charges. Therefore, if the intrinsic values of $\sigma_o$ are approximately constant on the outer surface of somatic membrane of molusc neurons, the calculated values of $\sigma'_o$ for sodium channel must be smaller than for calcium channel what is actually observed (Table 2). The same relation must hold and actually holds for the calculated $K_M$ values (Table 2).

Thus, these facts indicate that the distribution and properties of the functional groups responsible for the somatic membrane surface potential are generally universal. From above facts we see that the most probable candidate for these functional groups is the polar head group of phosphatidylserine. Our values of $K_{Ca} = 60\text{–}90\,\text{M}^{-1}$ and $K_{Mg} = 15\text{–}25\,\text{M}^{-1}$ are close to those obtained by Hammoudan et al. (1981) $K_{Ca} = 75\,\text{M}^{-1}$, $K_{Mg} = 20\,\text{M}^{-1}$ for PS vesicles and

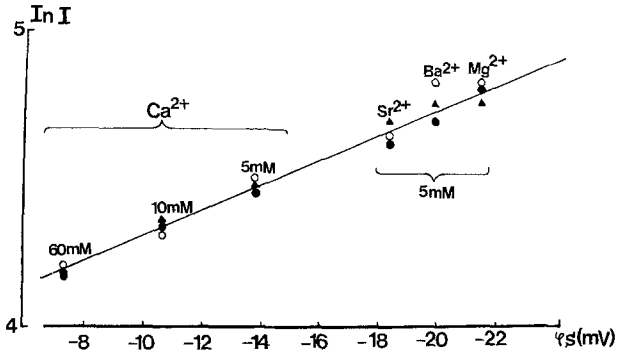


**Fig. 5.** Dependence of sodium conductance on surface potential. Points represent mean values for 3 cells. Straight line was drawn according to Eq. (20). The concentration and the type of divalent cation are indicated in the Figure

by Ohki and Kurland (1981) $K_{Ca}=30\,M^{-1}$, $K_{Mg}=10\,M^{-1}$ for PS monolayers. As to our value of $pK_H=6.2$, it can be compared with one of the values of $pK_H=6.3$ found by Hille et al., (1975). This value exceeds the value of $pK_H=3.3$ for PS (Ohki & Kurland, 1981). This discrepancy is probably caused by the process of monovalent ion binding to PS, since Ohki and Kurland (1981) showed that increase of $K_M$ from 0 to $0.6\,M^{-1}$ ($K_{Na}$) when $C_M \simeq 0.1\,M$ shifts the apparent value of $pK_H$ to 5.8, i.e. the value which is close to that observed in the present work.

For comparison with the results of this work, it should be mentioned that the outer surface of mammalian neurons has similar properties which are responsible for their surface potential (Kostyuk & Mironov, 1982). In particular, the values of $\sigma_o'=0.15\,e/nm^2$ and $K_{Ca}=70\pm10\,M^{-1}$ are close to the values obtained in the present work.

The maximal value of the steady-state sodium current $I_{Na}$, when the concentration of carrier (sodium) ions is constant in the bathing solutions, depends on the nature and concentration of divalent ions in extracellular solution (*see* Results and Figs. 1 and 2). If we assume that this value is linearly dependent upon the concentration of $Na^+$ ions near the outer membrane surface ($C_{Na}$), then from Eq. (15) we find

$$\ln I_{Na}=\ln(P_{Na}C_{Na})-\varphi F/RT \qquad (21)$$

where $P_{Na}$ is the effective permeability of the membrane for sodium. In fact, the value of $\ln I_{Na}$ increases in a sequence $Ca<Sr<Ba<Mg$, when they are present in extracellular solution in equimolar quantities. This sequence corresponds to the decreasing affinity of these ions to the functional groups of the membrane (Table 2). The value of the logarithm of the maximal amplitude of the sodium current correlates well with the value of the surface potential during both the changes in the concentration of $Ca^{2+}$ ions and the replacement of $Ca^{2+}$ ions by other divalent cations (Fig. 5). A similar effect has been observed by Ohki (1978) for the sodium current in the axonal membrane and by Ohmori and Yoshii (1977) for sodium and calcium currents of the tunicate egg cell membrane.

Thus, our data are consistent with the hypothesis that the value of steady-state sodium current is determined by the near-membrane concentration of the carrier ions. Therefore this effect has to be taken into account in determinations of such characteristics of ionic channels as permeability and dissociation constants of binding sites for ions bound to the functional groups of the ionic channels, because they are determined from the dependence of the amplitude of ionic currents on the concentration of the carrier ions.

Our data about the surface potential of the outer side of the mollusc neuronal membrane have been employed for the study of the energy profile of a calcium channel (Kostyuk, Mironov & Doroshenko, 1982).

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