Correction. In the article "The Electrostatic Basis of Mg++ Inhibition of Transmitter Release" by Robert U. Muller and Alan Finkelstein that appeared in the March 1974 issue of Proc. Nat. Acad. Sci. USA 71, 923–926, the authors request the following change. On page 924, the sentence beginning on the 19th line below the heading Predictions of the theory should read: Substituting this into Eq. 8, we find that $\Psi_0$ decreased by 1.7 mV (from $-80.2$ to $-78.5$ mV).

Authors' Statement

Over the last few months we have reported experiments about the existence and nature of "transfer factor." These experiments were performed both independently and jointly over a 10-month period by three people (1–3). Our preparation of biologically active material originally occurred with a success rate of 30% (20 successful preparations). More recently, however, since April, 1974, no member of the group has been able to prepare active material.

This has led us to be concerned that our original positive results may not have been obtained by the procedures described. We leave it to the kindness of our scientific colleagues to accept this statement of uncertainty and potential retraction with our sincere apologies.

David Dressler
Huntington Potter


The Electrostatic Basis of Mg++ Inhibition of Transmitter Release
(neuromuscular junction/quantal content/diffuse double layer/Mg++-Ca++ antagonism/Na+-Ca++ antagonism)

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ABSTRACT The inhibition by Mg++ of stimulus-evoked transmitter release is attributed to a decrease in surface potential, \(\psi_o\), on the outer surface of the presynaptic terminal and hence a lower surface calcium concentration, \([Ca^{++}]_o\). Data on the frog neuromuscular junction are quantitatively fit by assuming that there is a negative charge density, \(\sigma\), on the outer surface of the presynaptic terminal of \(6.5 \times 10^{13}\) charges per cm\(^2\) and that simple diffuse double layer theory is applicable. No specific binding of Mg++ or Ca++ is required. Without any additional assumptions, the inhibitory effect of univalent cations is also quantitatively predicted.

INTRODUCTION

The quantal release of acetylcholine (ACh) at the neuromuscular junction has received considerable attention, both because of its intrinsic interest and because of its relevance to transmitter release at autonomic neuroeffector endings and central synapses. It is generally accepted that ACh release results from the interaction of ACh-containing vesicles in the pre-synaptic terminals with the presynaptic plasma membrane, leading to exocytosis of transmitter (1). It is also widely held that stimulus-evoked release results from entry of Ca++ into the presynaptic terminals (2). Although some doubt still exists, we feel the evidence is sufficiently compelling to warrant acceptance of this picture.

A question of some interest in this context is why Mg++ inhibits stimulus-evoked release of transmitter (3). Proposed answers have focussed on postulated binding of Ca++ to release sites on the presynaptic membrane and competition between Ca++ and Mg++ for these sites (4, 5). We offer here an alternative explanation that involves no specific binding of ions, but depends simply on some general consequences of diffuse double layer theory. In particular, if a membrane bears a net negative charge, then the concentration of Ca++ near the membrane is not determined solely by its concentration far from the membrane (i.e., in “bulk” solution). Rather, because the total space charge near the membrane is constant, if the bulk concentration of any other cation (e.g., Mg++) is, for instance, increased, the surface concentration of Ca++ must decrease. Since it must be the ion concentration immediately at the membrane surface which is the “effective” concentration, it becomes a quantitative question whether our proposed mechanism is capable of accounting for the Mg++ inhibition of Ca++ activity. Although we consider experimental data from only one preparation—the frog neuromuscular junction—we believe that the same inhibitory mechanism operates at most, if not all, chemically transmitting synapses and at such hormone-releasing synapses as the chromaffin cells of the adrenal medulla and the pancreatic \(\beta\)-cells.

THE MODEL

Assumptions

1. For a presynaptic spike of given amplitude and duration, the quantal content (m) of the end plate potential is a function of \([Ca^{++}]_o\), the concentration Ca++ at the outer surface of the presynaptic terminal, as distinct from [Ca++], the concentration of Ca++ in the “bulk” extracellular fluid (or bath). The model we have in mind (although it is not necessary to accept this for our present considerations) is the one proposed by Katz and Miledi (6). Quantal content is determined by the entry of Ca++ into the presynaptic terminal during a presynaptic action potential. This entry occurs through voltage-dependent calcium channels in the presynaptic membrane. Thus, the calcium that the channels “see” is that at the surface.

2. The outer surface of the presynaptic membrane has a negative surface charge density \(\sigma\) (expressed in charges per unit area).

3. The concentration (in charges per unit volume) of a given ion at the membrane surface, \([i]_o\), is related to its concentration in the bath, \([i]_w\), through the Poisson-Boltzmann equation, i.e., diffuse double layer theory.

Theory

The basic premises of the theory are: (1) the concentration at a distance \(x\) from the surface of the terminal* of ionic species \(i_{\pm n}\) is given by the Boltzmann Distribution:

\[
[i_{\pm n}] = [i_{\pm n}]_w e^{\pm n \kappa \psi/kT}
\]

where,

\[
[i_{\pm n}]_w = \text{concentration of } i_{\pm n} \text{ at large distances from the surface}
\]

\(\pm n = \text{valence of the ion}\)

\(q = \text{charge on the electron}\)

\(k = \text{Boltzmann constant}\)

\(T = \text{temperature in degrees Kelvin}\)

\(\psi = \text{electrostatic potential at a point } x (\psi = 0)\)

and (2) the electrostatic potential, \(\psi\), satisfies Poisson’s equation:

* The radius of curvature of the motoneuron terminal is large enough that, for purposes of double layer theory, we can treat it as planar.
We are particularly concerned with the surface concentration of calcium, \([Ca^{++}]_o\), which is given from the Boltzmann distribution (Eq. 1) as:

\[
[Ca^{++}]_o = [Ca^{++}]_o e^{-\frac{2q\psi_o}{kT}}. \tag{8}
\]

Equations 7 and 8 are the relations needed for our calculations. Given \(\sigma\), which is empirically determined (as we shall see shortly), \(\psi_o\) is calculated from Eq. 7. (Note that \(\psi_o\) is a function of both univalent and divalent cation concentrations in the bulk solution.) This value is then substituted into Eq. 8 to give \([Ca^{++}]_o\), which can then be used to predict the change in quantal content, \(m\), through the relation between \(m\) and \([Ca^{++}]_o\) (assumption 1). For the frog neuromuscular junction at low concentrations of calcium, the relation:

\[
[m] \propto ([Ca^{++}]_o)^{1.8}. \tag{9}
\]

(Dodge and Rahamimoff (5) experimentally established the relation:

\[
[m] \propto ([Ca^{++}]_o)^{1.8}.
\]

The conditions of the experiments were such, however, that \([Ca^{++}]_o\) should have varied almost linearly with \([Ca^{++}]_o\), and hence relation 9 is valid.)

**Predictions of the theory**

1. Qualitative aspects—Before discussing quantitative aspects of the theory, we note that the dependence of quantal content on Mg\(^{++}\) and Na\(^+\) concentrations is in obvious *qualitative* accord with theory. The reduction of quantal content by increased Mg\(^{++}\) concentration (3, 5) results from a decreased\(^\S\) surface potential, \(\psi_o\), and hence a lower surface calcium concentration ([Ca\(^{++}\)]. Similarly, the increase in quantal content upon replacement of Na\(^+\) by sucrose (8, 9) is explained by an increase in \(\psi_o\), produced by a lower univalent cation concentration, and hence an increase in [Ca\(^{++}\)].

2. Quantative aspects—The data points in Fig. 1 reproduce the results from Fig. 5A of Dodge and Rahamimoff (5) showing the reduction in end plate potential (e.p.p.) with increasing Mg\(^{++}\) concentration. In going from 0.5 mM Mg\(^{++}\) in the presence of 0.4 mM Ca\(^{++}\) and 116 mM NaCl (+2 mM KCl) to 1.0 mM Mg\(^{++}\), they observed that the e.p.p. (which is proportional to \(m\)) decreased by a factor of 1.65. Our theory demands (Eq. 9) that [Ca\(^{++}\)] must have decreased by a factor of 1.14. Substituting this into Eq. 8, we find that \(\psi_o\) decreased by 1.5 mV (From −76.8 to −75.3 mV). With this information we then obtain from Eq. 7 that:

\[
\sigma = 6.5 \times 10^{14} \text{ charges cm}^{-2}
\]

or

\[
\sigma = 1 \text{ charge per } 154 \text{ Å}^2. \tag{10}
\]

Using this value of \(\sigma\), we generate the theoretical curve of Fig. 1, which fits all of the data points closely.

\(^\dagger\) We assume that the amount of charge within the membrane is so small that it is effectively approximated by zero; thus there is no space-charge region within the membrane. Also, we neglect the small contribution to \(\sigma\) that results from the potential difference across the membrane.

\(^\S\) We are referring to the *absolute* value of the surface potential.

\(^\dagger\) We confine our analysis to the frog neuromuscular junction at calcium concentrations where relation 9 is observed. This offers the best quantitative data with which to compare the theory.
With this same value of \( \sigma \), we can also fit other data. From Fig. 3B of Dodge and Rahaminoff, an increase of Mg\(^{++}\) concentration from 0.5 mM to 2 mM shifts the plot of log e.p.p. versus log \([\text{Ca}^{++}]\) by a factor of 1.55 along the \text{Ca}^{++} axis; we predict a factor of 1.47. From the same figure, a further increase of Mg\(^{++}\) concentration from 2 mM to 4 mM shifts the curve along the \text{Ca}^{++} axis by a factor of 1.26, whereas we predict a factor of 1.37. The agreement of theory with experiment is reasonable.

It is also possible, without any additional assumptions, to predict the reduction in quantal content that occurs when univalent cation concentration is increased. Misler and Hurlbut (unpublished observations) have measured the changes in quantal content that occur when Na\(^{+}\) is maintained at about 64 mM (K\(^{+}\) = 2 mM), and glucosamine is isotonically substituted for sucrose. Their results are presented in Table 1 along with the values predicted from the theory; the agreement is quite satisfactory.

**DISCUSSION**

**Comments on the assumptions of the model**

We have shown that the effects of Mg\(^{++}\) and univalent cation concentration on quantal content at the frog neuromuscular junction are quantitatively predicted from the assumption that there exists a negative surface charge of a given magnitude (6.5 \times 10^{-10} \text{ charges per cm}^2) on the outer surface of the presynaptic terminal.

A negative surface charge has been found on all cells investigated to date, including nerve, and probably accounts both from acidic phospholipids and from proteins associated with the plasma membrane and its extraneous coats. In addition, material in the synaptic cleft may contain negative charged groups, and it is possible that these charges are formally included in the surface charge density, \( \sigma \).

By focussing attention on diffuse double layer theory, we exclude specific adsorption of ions, ion size, binding of ions to specific charge groups on the membrane, etc. The only relevant property of the ions is their valence and concentration, and the precise chemical nature and spatial distribution of the charges contributing to \( \sigma \) are not relevant. Obviously, these factors may play a role and lead to quantitative modifications of the results. The good agreement between the theory we present and the available data does indicate that to at least a first approximation, simple double layer theory, without these other refinements, adequately treats the major aspects of the phenomena. We recognize, however, that simple electrostatic screening, which quantitatively accounts for the action of Mg\(^{++}\), is not sufficient to explain entirely the effects of other multivalent cations. Although we feel that Sr\(^{++}\) and Ba\(^{++}\) behave like Mg\(^{++}\) on the outside of the presynaptic terminal to reduce quantal content, their presence on the outside, after entry into the terminal through the calcium channels, acts to increase quantal content; this complicates the interpretation of data obtained with these ions (11, 12).

The experimental data are taken from the unpublished results of Misler and Hurlbut on the neuromuscular junction of frog cutaneous pectoris. All media (pH 6.6) contained approximately (see footnotes) 64 mM Na\(^{+}\), 2 mM K\(^{+}\), and the concentrations of Ca\(^{++}\) and Mg\(^{++}\) shown in the table. Univalent cation concentration was changed from approximately (see footnotes) 66 mM to 118 mM by isotonic substitution of glucosamine chloride for sucrose. Glucosamine\(^+\) concentration was calculated from the pK \( \approx 7.6 \) of glucosamine.) The theoretical values of the ratio of quantal content in 66 mM (\( m_{a6} \)) to quantal content in 118 mM (\( m_{a18} \)) were calculated from Eqs. 7, 8, and 9 with \( \sigma = 6.5 \times 10^{-10} \text{ charges cm}^{-2} \times (dT/2\pi) = 1.36 \times 10^{12} \text{ (charges)}^3 \text{ cm}^{-3} \text{ mol}^{-1} \). The numbers in parentheses in the third column give the number of experiments.

* Actual values of \([i^+]\) were 62.8 mM and 114.4 mM.
† Actual values of \([i^+]\) were 67.3 mM and 119 mM.
‡ Actual values of \([i^+]\) were 68.8 mM and 120.4 mM.

**Comparison with the binding theory**

The binding theory attributes the reduction in quantal content produced by Mg\(^{++}\) and Na\(^{+}\) to competition of these ions with Ca\(^{++}\) for presumptive release sites (4, 5, 10). Although physically quite different, the two theories are formally very similar. Instead of competitive binding leading to displacement of Ca\(^{++}\) from the membrane, we attribute the effects of Mg\(^{++}\) and univalent cations to reduction in the surface potential, and hence a reduction in surface Ca\(^{++}\) concentration. In choosing between these two theories one can, at present, only be guided by the simplicity of the assumptions. To account for the data, the binding theory must postulate ad hoc binding sites and must invoke empirically determined binding constants for Ca\(^{++}\), Mg\(^{++}\), and univalent cations. In contrast, we have shown that the effects of Mg\(^{++}\) and univalent cations arise from simple physical theory and are predicted from one empirically determined constant, the surface charge density, \( \sigma \), on the outer membrane surface. The value of \( \sigma \), incidentally, is comparable to other estimates of negative surface charge densities on the outer surface of nerve membranes (15, 16).

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### Table 1. The effect of univalent cation concentration on quantal content (m) at constant calcium and magnesium concentrations

<table>
<thead>
<tr>
<th>[Ca(^{++})] (mM)</th>
<th>[Mg(^{++})] (mM)</th>
<th>( m_{a6}/m_{a18} ) (experimental)</th>
<th>( m_{a6}/m_{a18} ) (theoretical)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>4.05</td>
<td>3.94*(8)</td>
<td>4.0*</td>
</tr>
<tr>
<td>0.26</td>
<td>1.03</td>
<td>7.34*(8)</td>
<td>10.0†</td>
</tr>
<tr>
<td>0.26</td>
<td>0.0</td>
<td>22†(2)</td>
<td>28.8‡</td>
</tr>
</tbody>
</table>

The values in parentheses are the third column gives the number of experiments.

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\* If Na\(^{+}\) is substituted for sucrose, the height of the presynaptic action potential will increase, and this might affect m. The point of these experiments is that ionic strength is changed at constant Na\(^{+}\) concentration, thus circumventing this problem.

\* Actually, in terms of the model, \( \sigma \) is the charge density on the outer surface in the vicinity of the calcium channels. This might differ from the charge density at other points on the terminal.

\†† It is more appropriate to use ion activities rather than concentrations in Eq. 7. This leads to a somewhat smaller value for \( \sigma \). Because of the uncertainties in calculating single ion activities, however, we do not think it useful to introduce this refinement.


