J. Membrane Biol. 58, 115–121 (1981)

The Journal of
Membrane Biology

Competitive Membrane Adsorption of Na^+, K^+, and Ca^{2+} in Smooth Muscle Cells

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Summary. A theory for Na^+, K^+ and Ca^{2+} competitive adsorption to a charged membrane is used to explain a number of experimental observations in smooth muscle. Adsorption is described by Langmuir isotherms for mono- and divalent cations which in turn are coupled in a self-consistent way to the bulk solution through the diffuse double layer theory and the Boltzman equations. We found that the dissociation constants for binding of Na^+, K^+ and Ca^{2+} in guinea pig taenia coli are *ca.* 0.009, 1.0, and 4 $\times 10^{-8}$ M, respectively. Furthermore, the effect of a Ca^{2+} pump that maintains free surface Ca^{2+} concentration constant is investigated. A decrease in intracellular Na^+ content results in an increased Ca^{2+} uptake; part of this uptake is due to an increase in surface-bound Ca^{2+} in an intracellular compartment which is in contact with the myofilaments. Variations in the amount of charge available to bind Ca^{2+} and the surface charge density are studied and their effect interpreted in terms of different pharmacological agents.

Mechanical activity of smooth muscle is regulated by the cytoplasmic concentration of calcium ions [42]. This free Ca^{2+} concentration in turn is controlled by membrane Ca^{2+} pumps, channels, and binding sites which cause local shifts in Ca^{2+} content [7, 43]. Research into these processes is entering into a new era of quantitation owing to developments in techniques of measuring Ca^{2+} in cells and their components [11, 44, 45]. At the same time, there is a surge of new discoveries related to the Ca^{2+}-sensitive components of the contractile proteins [2, 31]. As a consequence, data describing fluctuations in cell Ca^{2+} which accompany mechanical activity and Ca^{2+} binding to components of the contractile apparatus and cell membranes are now appearing in the literature [10, 17, 19]. It is therefore surprising that, to date, no serious attempt has been made to develop a theoretical framework based on physical-chemical theory which will account for fluctuations in smooth muscle cell Ca^{2+}. Of particular importance to medicine are the Ca^{2+} fluctuations brought about by changes in Na^+ metabolism [20].

In this presentation, we have sought to explain some Na^+, K^+, Ca^{2+} interactions on the basis of their interplay with a negatively charged membrane surface. If the intracellular membrane contains a negative charge density then, as shown in other physiological systems [12, 21], ion adsorption and increased surface ionic concentrations will affect the membrane surface potential and hence the movement of ions across the membrane [*cf.* 21]. Clearly, an understanding of adsorption at membrane surfaces is important in understanding membrane transport phenomena.

The effect of adsorption on ionic transport has been studied in model membrane systems [3, 24, 30]. However, in almost all cases [4, 18, 28, 29], adsorption has been described with the simple Langmuir adsorption isotherm. If more than one ionic specie is present, the multi-ionic effects are either coupled through screening by way of the double layer theory [23, 38] or mixing (entropic) effects are ignored in the adsorption isotherm [35]. In the first section, we present a self-consistent theory of Na^+, K^+ Ca^{2+} competitive adsorption to a charged surface in a closed system. The K^+ has been included to more closely mimic physiological conditions. In the second and final sections, we show, using reasonable parameters for binding, that a number of experimental observations in smooth muscle can be explained by the adsorption theory.

Theory

Although membrane Ca^{2+} binding proteins have been isolated in a number of systems [*cf.* 13, 15], they

0022–2631/81/0058–0115 $01.40

have not yet been isolated in smooth muscle. The evidence for such membrane binding sites in smooth muscle is therefore indirect [*cf.* 9] and inferred from experiments that show the existence of intracellular Ca^{2+} stores that are affected by extracellular pharmacological agents. For the purpose of this paper, we will assume such membrane sites exist. Within the framework of this assumption it follows that these sites must have a high Ca^{2+} affinity so that intracellular concentrations are maintained below levels that can induce contractions ($<10^{-7}$ M). It is reasonable that these proteins are homologous to the ones studied (e.g., TnC and MCBP [39]) in their multidentate helix-loop-helix structure. For such proteins, multiple anionic sites are available for desolvation and coordination of the Ca^{2+}. It has been found that the active Ca^{2+} site [27] in troponin-C (TnC) has a Ca^{2+} affinity of approximately 10^{-6} M [37], and Mg^{2+} does not compete with Ca^{2+} at this site. Therefore, the membrane protein will be assumed to contain multiple (two) anionic binding sites to which one Ca^{2+} or multiple monovalent cations can bind, and these units are assumed to be sufficiently isolated so that no crossbridging occurs. With this model a system of equations can be presented that are readily solved self-consistently to yield the equilibrium properties between an electrolyte solution containing Na^+, K^+ and Ca^{2+} and a charged membrane surface. Generally, this calculation consists of satisfying a modified Gouy-Chapman equation, Boltzman equations, and Langmuir adsorption isotherms for a particular set of parameters that describes the system's properties [*cf.* 28, 29].

The modified Gouy-Chapman equation assumes the surface charge is smeared uniformly over the surface and couples this free charge density (σ_f) with the bulk electrolyte concentrations. For the system in which Na^+, K^+ and Ca^{2+} are in solution, the modified Gouy-Chapman equation is [*cf.* 4],

$$\sigma_f = (2\varepsilon_r \varepsilon_0 kT\{[Ca]^i_\infty (e^{-2e\psi(0)/kT} + 2e^{+e\psi(0)/kT} - 3) + ([Na]^i_\infty + [K]^i_\infty)(e^{-e\psi(0)/kT} + e^{e\psi(0)/kT} - 2)\})^{\frac{1}{2}}, \quad (1)$$

where σ_f is given by the total charge density (σ) minus the amount of Na^+ bound (σ_n), K^+ bound (σ_k) and twice the amount of Ca^{2+} bound ($2\sigma_c$). The symbols ε_r, ε_0, k, T, e and $\psi(0)$ represent the dielectric constant, permittivity of free space, the Boltzman constant, the absolute temperature, the electronic charge, and the surface potential, respectively. The free intracellular concentrations for Na^+, K^+, and Ca^{2+} are represented by $[Na]^i_\infty$, $[K]^i_\infty$, and $[Ca]^i_\infty$, respectively.

The Boltzman equation describes how the electrolyte distributes at a distance x from a planar surface and is given by

$$c^i(x) = c^i_\infty e^{-z_i e\psi(x)/kT}, \quad (2)$$

where $c^i(x)$ is the concentration of the ion of choice (valence z_i) a distance x from the membrane.

Finally, the Langmuir adsorption isotherms describe the equilibrium between the adsorbed ions at fixed sites and the surface concentration ($M(0)$ and $D(0)$; monovalent and divalent, respectively). For the j^{th} monovalent (M_j) the isotherm is given by [*cf.* 4]

$$M_j(0) = \frac{K_D^{M_j}\sigma_{M_j}}{\sigma - (2\sigma_D + \sigma_{M_1} + \sigma_{M_2})} \quad (3a)$$

while for the divalent cation the Langmuir adsorption isotherm for binding to noncrossbridging units is given by (M.A. Kolber, *submitted for publication*)

$$D(0) = \frac{2K_D^D\sigma_D(\sigma - 2\sigma_D)}{(\sigma - (2\sigma_D + \sigma_{M_1} + \sigma_{M_2}))^2} \quad (3b)$$

where K_D^M and K_D^D are the dissociation constants for the monovalent and divalent cations.

For Na^+, K^+ and Ca^{2+} in solution, Eqs. (1) through (3) constitute a system of seven equations. When the total surface charge (σ), the dissociation constants (K_D^N, K_D^K, K_D^C: Na^+, K^+, Ca^{2+}), temperature (T), and internal concentrations ($[Ca]^i_\infty$, $[Na]^i_\infty$, $[K]^i_\infty$) are specified the amount of each cation adsorbed to the surface (σ_n, σ_k, σ_c) the surface potential ($\psi(0)$) and the free surface concentrations ([Na(0)], [K(0)], [Ca(0)]) can be found by solving the equations self-consistently. In a closed system the total concentrations are given by the sum of the free and bound fractions (M.A. Kolber, *submitted for publication*). The results of such a self-consistent calculation in a closed system is described under various conditions in the following section.

Results

In Fig. 1, variations in internal free (bulk) Ca^{2+} are shown as a function of total Ca^{2+} for different free internal Na^+ concentrations (which are approximately equal to the total Na^+ concentrations) and fixed free K^+ concentrations (140 mM). The dots represent a dissociation constant for K^+ binding (K_D^K) of 0.2 M, while the solid lines are for $K_D^K = 0.05$ M. As expected, a lower K_D^K indicates more K^+ is being bound, and it takes less total Ca^{2+} to produce a given free Ca^{2+}. Only a small fraction of the monovalents are bound relative to their aqueous concentrations so that changes in screening due to binding of one fraction are small. Raising the free Na^+ at a fixed total Ca^{2+}

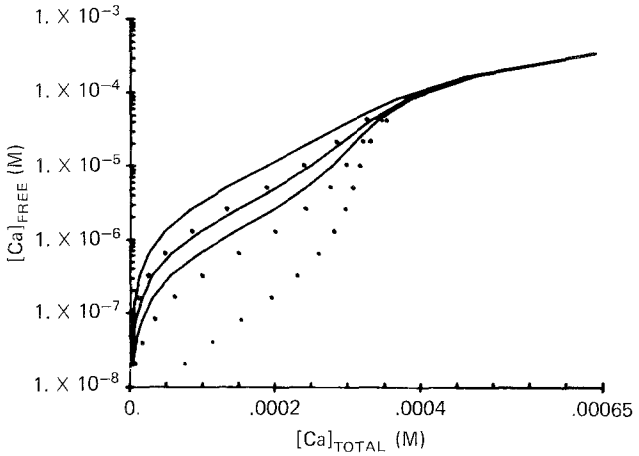

Fig. 1. The internal free Ca^{2+} is plotted against the total Ca^{2+} content for different internal free Na^+ concentrations at fixed free K^+ (0.14 M) for K_D^K equal to 0.2 M (dotted lines) and 0.05 M (solid lines). For each K_D^K value, three Na^+ curves (0.05 M, 0.018 M, 0.003 M) are shown; the leftmost curve in each set corresponds to $[Na]_\infty^+ = 0.05$ M, decreasing sequentially to $[Na]_\infty^+ = 0.003$ M for the rightmost curve. The curves were calculated using: $T = 37$ °C, $\sigma = 0.003$ electronic charges/Å^2, $K_D^C = 10^{-7}$ M, $K_D^N = 10^{-2}$ M and cell vol/area $= 0.8 \times 10^{-4}$ cm

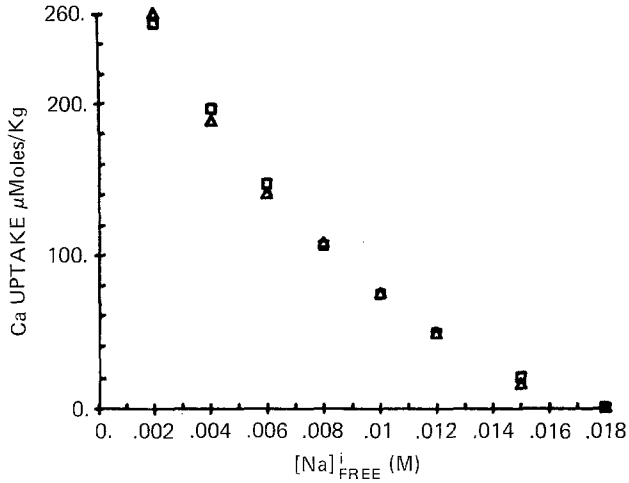

Fig. 2. The Ca^{2+} uptake given is shown as a function of free cytoplasmic Na^+ at fixed K^+ (0.14 M). The triangles (△) represent data (P. Aaronson & C. van Breemen, *submitted for publication*) and the squares (□) the fit using $T = 37$ °C, $\sigma = 0.005$ electronic charges/Å^2, $K_D^C = 4 \times 10^{-8}$ M, $K_D^N = 0.009$ M, $K_D^K = 1.0$ M and cell vol/area $= 0.8 \times 10^{-4}$ cm. The internal free Ca^{2+} concentration was fixed at 5×10^{-8} M

is shown (Fig. 1) to result in an increase in a free Ca^{2+}. This is due to a decrease in bound Ca^{2+} which results from an increase in monovalent screening and decrease in bound Ca^{2+} due to competition. Since Eqs. (1) to (3) possess a symmetry in K^+ and Na^+, any changes in Ca^{2+} that result from variations in a parameter of one of the monovalents could be produced by changes in the same parameter of the other monovalent. Therefore, Fig. 1 can be used to indicate what effects varying K_D^N and free K^+ would have on the Ca^{2+} curves.

As the free Na^+ content is increased (Fig. 1), less total Ca^{2+} content is necessary to produce a specific free Ca^{2+} concentration. This point is shown more clearly in Fig. 2 where we have varied the dissociation constants and surface charge in order to get a reasonable (by eye) fit to the experimental Ca^{2+} uptake data that was obtained from the taenia coli (P. Aaronson and C. van Breemen, *submitted for publication*). The parameters used to fit the data are $K_D^N = 0.009$ M, $K_D^K = 1.0$ M, $K_D^C = 4 \times 10^{-8}$ M, $\sigma = 0.005 e/$Å^2, cell vol/area $\simeq 0.8 \times 10^{-4}$ cm [22], $T = 37$ °C, and the intracellular Ca^{2+} was fixed at 5.0×10^{-8} M. The compartment in which we are considering binding to occur (indicated by the cell vol/area ratio) is the internal plasma membrane surface, exclusive of binding fractions such as the sarcoplasmic reticulum (SR). The fit in Fig. 2 is good, but not unexpected from the number of variables (four). However, these values are within the expected physiological range found in other biological systems [13, 21, 22, 33, 36]. The value of K_D^C is significantly lower than 10^{-6}, the dissociation constant for the low affinity Ca^{2+} binding site. One explanation is that the K_D for the binding protein was obtained assuming a hyperbolic relationship (Michaelis-Menten) [37]. When such a relationship is assumed, the K_D obtained is larger than that when Eq. (3*b*) is used (M.A. Kolber, *submitted for publication*). For the fit values used, the total Ca^{2+} contained in this compartment at $[Na]_\infty^i \simeq 0.018$ M is 77 μM Ca^{2+}/kg tissue. This means that for the cell to remain in a relaxed state, a large fraction of the total Ca^{2+} would have to be bound.

In Fig. 3, we show how the free charge density varies as a function of $[Na]_\infty^i$ (solid curve). The peak in the free charge density is a consequence of two factors: (1) screening and (2) competition. As $[Na]^i$ is increased from zero, the Ca^{2+} bound fraction is decreased due to increased Na^+ binding and screening. However, the amount of Ca^{2+} unbound is not compensated by these effects until higher Na^+. For high $[K^+]^i$, the free charge density divided by $\sqrt{[Na]_\infty^i + [K]_\infty^i}$ is approximately proportional to $\sinh(e\psi(0)/2kT)$, which is shown as the dashed line (Fig. 3). This effect is not realized when Ca^{2+} can bind to the sites in a one-for-one stoichiometry. The peak is a sensitive function of the parameters, decreasing and moving to higher $[Na]^i$ values with an increase in K_D^N and/or K_D^K. As K_D^N is lowered, the peak

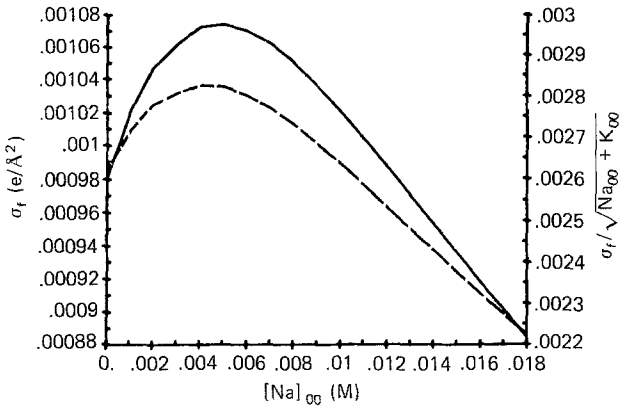

Fig. 3. The free charge density (solid line) is plotted on the left y axis as a function of free internal Na^+. The curve for $\sigma_f/\sqrt{Na_\infty + K_\infty}$ (dashed line; right y axis) is plotted for comparison against the free internal Na^+. The free internal K^+ and Ca^{2+} were fixed at 0.14 M and 5×10^{-8} M, respectively, while the parameter values were held at the values determined in Fig. 2

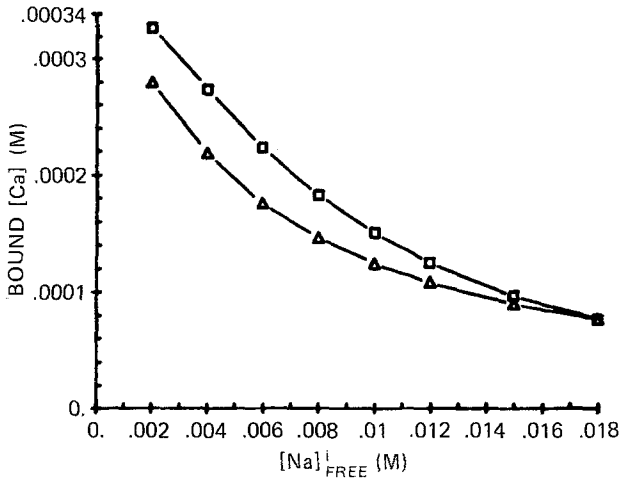

Fig. 4. The effective bound concentration of Ca^{2+} ($\equiv \sigma_c/(N_{av} \cdot \text{vol})$; N_{av} = Avagadros number) as calculated from the theory is plotted against the free internal Na^+. The top curve (□) was calculated using the same parameter values as in Fig. 3 at a fixed internal Ca^{2+} concentration of 5×10^{-8} M. The lower curve (△) was calculated using the parameter values in Fig. 3; however, in this case the free internal Ca^{2+} was allowed to vary so that the free surface Ca^{2+} concentration was fixed at the value determined by $[Ca^{2+}]^i_{free} = 5 \times 10^{-8}$ M, $[Na^+]^i_{free} = 0.018$ M and $[K^+]^i_{free} = 0.14$ M yielding $[Ca(0)]^i_{free} \cong 1.59 \times 10^{-7}$ M

moves to lower $[Na]^i$ values; however, the height of the peak eventually diminishes as $[Na]^i$ approaches zero.

In Fig. 4 is shown the effective concentration of bound Ca^{2+} ($\equiv \sigma_c/(N_{av} \cdot \text{vol})$; N_{av} = Avagadros number) as a function of free cytoplasmic Na^+. The top curve (connected squares) was calculated assuming the internal free (bulk) Ca^{2+} concentration remained fixed, while the bottom curve was calculated assuming the free Ca^{2+} concentration at the membrane was constant. The top curve represents a situation in which the cell maintains a low free Ca^{2+} concentration by binding to the membrane. The bottom curve represents a situation in which the Ca^{2+} that enters the cell is removed by a mechanism that senses only the Ca^{2+} at the membrane surface. If we assume that the Ca^{2+} pumps that exist (e.g., in the SR) are activated by increases in the free surface Ca^{2+} and work to keep this surface concentration constant, then these pumps may provide a milieu described by the bottom curve of Fig. 4. This assumption is in line with the thinking [26] that the pumping action is a consequence of a conformational change in the protein, which produces a high energy metastable state allowing the pumped molecule to move against its gradient. In such a model, the probability that a molecule is bound is proportional to the probability it will be pumped. It is reasonable to expect the physical binding state to lie somewhere between the curves of Fig. 4. It is noteworthy that in both cases in Fig. 4 the bound Ca^{2+} store, which is in contact with the intracellular space, increases as Na^+ declines.

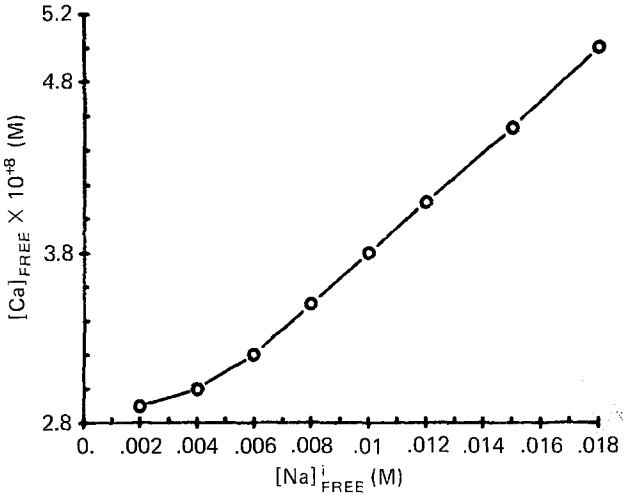

Fig. 5. Using the fit parameters of Fig. 3, we display the calculated effect of varying the internal free Na^+ concentration on the internal free Ca^{2+} concentration when the free surface Ca^{2+} concentration is held fixed at 1.59×10^{-7} M

Ca^{2+} efflux experiments, following an established protocol [1], indicated a slow component that responded to a lowering of $[Na]^e$ with an increase in size and decrease in rate. This increase in size could be due to the increased amount of binding shown in Fig. 4. If the free surface Ca^{2+} is maintained constant as $[Na]^i$ is varied, the free internal (bulk) Ca^{2+} must change to accommodate these conditions. In Fig. 5 is

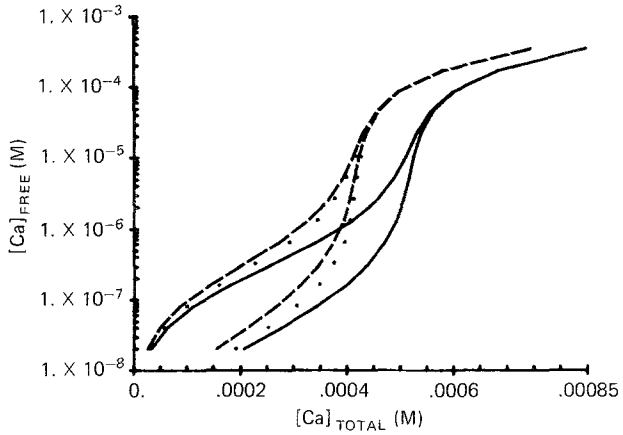

Fig. 6. $[Ca^{2+}]_{free}$ is plotted against $[Ca^{2+}]_{total}$. The solid curves are for $0.005\,e/Å^2$ total charge, no inaccessible charge; the dotted curves are for $0.005\,e/Å^2$ total charge, $0.001\,e/Å^2$ inaccessible to binding; and the dashed curves are for $0.004\,e/Å^2$, no inaccessible charge. In each set of curves, the right-hand curve is for $[Na^+]_{free} = 0.003$ M and the left-hand curve for $[Na^+]_{free} = 0.018$ M. The dissociation constants were kept as determined in Fig. 2, and $[K^+]_{free} = 0.14$ M

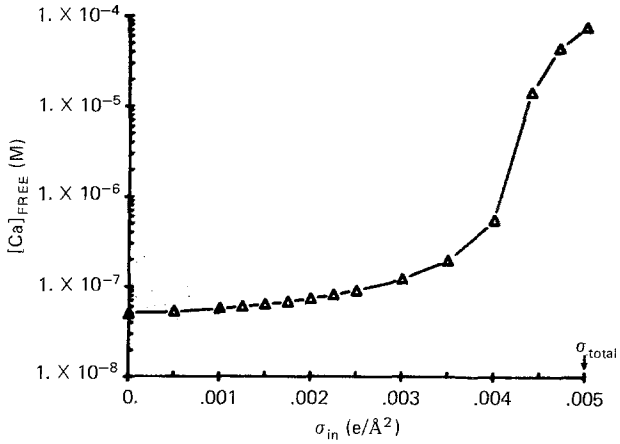

Fig. 7. $[Ca^{2+}]_{free}$ is plotted as a function of inaccessible charge (σ_{in}) when the total charge density is fixed at $0.005\,e/Å^2$ and the total Ca^{2+} content is fixed at 7.6×10^{-5} M. $[Na^+]_{free} = 0.018$, $[K^+] = 0.14$, and the remaining values are fixed as in Fig. 3

shown how $[Ca]^i$ changes, if [Ca(0)] is constant, as a function of $[Na]^i$. The free internal Ca^{2+} is reduced due to the decrease in competition and screening as $[Na]^i$ is removed. If no mechanism for maintaining the free surface Ca^{2+} is present and all the Ca^{2+} is sequestered by the surface, then the effective bound Na^+ and Ca^{2+} will vary as determined from the potential (*see* Fig. 3 dashed curve) as $[Na]^i$ is varied. Although only a small fraction (<5%) of the free Na^+ and K^+ concentration is bound, it can constitute a significant fraction (75%) of the total binding sites.

In Fig. 6 we show the effect of varying the surface charge. The solid lines are with the total surface charge (σ) equal to $0.005\,e/Å^2$ and the dashed curve is with $\sigma = 0.004\,e/Å^2$. The curves show a shift to lower total $[Ca^{2+}]$ as σ is decreased. The dotted curve represents a situation in which the total charge density is $0.005\,e/Å^2$, but only $0.004\,e/Å^2$ is available to binding while the remaining fraction ($0.001\,e/Å^2$) is inaccessible to binding. At high $[Ca]_{total}$, the dotted curve approaches the dashed curve while at low $[Ca]_{total}$ it approaches the solid curve. An inaccessible charge fraction, in general, would exist since there are numerous anionic residues with K_D's higher than those we are considering. If we fix the total Ca^{2+} content of our cell and investigate the effect of varying the amount of inaccessible charge on free $[Ca^{2+}]$ then Fig. 7 is obtained. In Fig. 7, we see that the available surface charge ($\sigma - \sigma_{in}$) will buffer the free Ca^{2+} until the inaccessible charge region gets so large that Ca^{2+} must be released into the cytoplasm. The significance of this buffering capacity will be discussed in the following section.

Discussion

In the previous sections, we have studied the effect of Na^+, K^+ and Ca^{2+} competitive adsorption to a charged surface. This calculation shows that variations of cytoplasmic Ca^{2+}, as a result of changes in intracellular Na^+, can be explained in terms of binding to a charged surface using physical parameters. The only calculation, until now, that has attempted to explain variations in cytoplasmic Ca^{2+} as a function of Na^+ concentration is the Na^+, Ca^{2+} exchange carrier [6, 32, 40, 41]. In this model, the concentration of intracellular Ca^{2+} is coupled to the Na^+ gradient. Experimental evidence has been accumulating in smooth muscle systems that indicates that the existence of a Na^+, Ca^{2+} exchange carrier in plasmalemma is improbable [9]. Furthermore, the functional form of the exchange carrier does not provide an adequate mathematical base from which to describe the physical complexity of the smooth muscle system. We have used theories that have been satisfactorily applied in both biological [12, 21] and model membrane [3, 18, 29, 38] systems. Double layer theory, though ignoring discreteness of charge effects [5, 14, 34], is a reasonable starting point for understanding surface charge effects. Not only does smeared charge theory allow for screening effects, it also permits one to mathematically couple bulk screening to adsorption theory in a self-consistent, relatively simple way.

In general, a simple system which maintains the dominant physics of the system has major advantages as a base from which to work. In regard to the present work, we see that ionic competitive adsorption is important in regulating the free ionic surface concentrations, which may be relevant in transmembrane calculations. Enhancement of surface concentrations will certainly be important in both passive and active transport of ions across membranes. Ionic binding to pumps, located in the membrane, is necessarily dependent upon the concentration of the binding ion at the interaction site. If these pumps in the membrane function to maintain a *local* concentration of ions, as described in the Results section, then intracellular Ca^{2+} and bound surface charge would reflect these as variations in intracellular Na^+ occur. As regards passive transport, the concentration of ions at the surface would not only affect binding at passive transport sites but the potential drop across the membrane. Even though we have not coupled nonequilibrium (flux) effects to our calculation, the adsorption calculation is a first step and does provide explanations for a number of experimental observations.

One of these observations is illustrated in Fig. 2. Both cytoplasmic Na^+ concentration and cellular Ca^{2+} content have been experimentally determined during a series of extracellular Na^+ substitutions with sucrose (P. Aaronson & C. van Breemen. *submitted for publication*). These experimental points could be fitted with the above theoretical treatment assuming "reasonable" values for both ion binding and membrane charge density. Attempts to fit these same data with the Na^+, Ca^{2+} exchange carrier model did not meet with success.

Our theory would be of still greater interest to smooth muscle physiology if it could lead to explanations for regulation of mechanical activity. There seem to be at least two suggestions for its applicability in this respect.

There exists substantial evidence that receptor activation causes the release of a smooth muscle cell Ca^{2+} fraction which is distinct from the Ca^{2+} sink which operates during relaxation [16]. Since this receptor-linked Ca^{2+} store must be replenished from the extracellular space, it was assumed to be localized near or on the inner surface of the plasmalemma. If we further assume that this Ca^{2+} store has the properties of bound Ca^{2+} in our model, then Fig. 7 would illustrate a Ca^{2+} release when receptor occupation decreased the availability of binding sites (i.e., increase σ_{in}).

Figure 7 indicates that intracellular Ca^{2+} release due to agonists would be buffered until the amount needed to be bound was greater than the available binding sites. At this point, a large increase in free Ca^{2+} would ensue, and unless an alternative store existed for the Ca^{2+} a contraction would follow. Furthermore, if two (or more) agonists share the same receptor pool, then application of either agonist would potentiate the action of the other. This has, in fact, been recently seen in rabbit aortic smooth muscle [46] where a concentration of prostaglandin E that does not elicit a contraction was used to enhance the contractions of submaximal concentrations of norepinephrine or angiotensin III.

A final possible example concerns vasodilation induced by sodium nitroprusside. The mechanism of this relaxation does not fall within any of the presently accepted theories, namely (1) Ca^{2+} antagonism or inhibition of Ca^{2+} influx; (2) stimulation of Ca^{2+} extrusion; (3) hyperpolarization; (4) elevation of cAMP levels [8, 25]. It has been suggested that sodium nitroprusside, being a large anion with low charge density, may increase the negativity of intracellular membrane surfaces and thus enhance divalent cation binding [8]. In that case, the reduction of cytoplasmic Ca^{2+} would be due to increases in surface charge density as illustrated in Fig. 6.

In conclusion, it should be emphasized that the above report is a first attempt to account for smooth muscle Ca^{2+} distribution by using a physical-chemical theory which can be shown to be relevant to a number of biological phenomena and which will be employed in future developments of kinetic models of transmembrane Ca^{2+} transport.

This work was supported by NIH Grants GM07028, HL25395. One of the authors (MAK) would like to thank Dr. Aaronson for results and discussions of his work prior to publication. The operation and continuing development of the PROPHET system is sponsored by the Chemical/Biological Information-Handling Program, Division of Research Resources, NIH.

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Received 6 May 1980; revised 19 September 1980