Modulation of Voltage-dependent Sodium and Potassium Currents by Charged Amphiphiles in Cardiac Ventricular Myocytes

Effects via Modification of Surface Potential

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ABSTRACT Modulation of voltage-dependent sodium and potassium currents by charged amphiphiles was investigated in cardiac ventricular myocytes using the patch-clamp technique. Negatively charged sodium dodecylsulfate (SDS) increased amplitude of $I_{Na}$, whereas positively charged dodecytrimethylammonium (DDTMA) decreased $I_{Na}$. Furthermore, SDS shifted the steady-state activation and inactivation of $I_{Na}$ in the negative direction, whereas DDTMA shifted the curves in the opposite direction. These shifts provided an explanation for the changes in current amplitude. Activation and inactivation kinetics of $I_{Na}$ were accelerated by SDS but slowed by DDTMA. These changes in both steady-state gating and kinetics of $I_{Na}$ are consistent with a decrease of the intramembrane field by SDS and an increase of the field by DDTMA due to an alteration of surface potential after their insertion into the outer monolayer of the sarcolemma. The effect of SDS on the steady-state inactivation of $I_{Na}$ was concentration dependent and partially reversed by screening surface charges with increased extracellular $[Ca^{2+}]$. These amphiphiles also altered the activation of the delayed rectifier K⁺ current ($I_{K,del}$), producing a shift in the negative direction by SDS but in the positive direction by DDTMA. These results suggest that the insertion of charged amphiphiles into the cell membrane alters the behavior of voltage-dependent $I_{Na}$ and $I_{K,del}$ by changing the surface charge density, and consequently the surface potential and implies, although indirectly, that the lipid surface charges are important to the voltage-dependent gating of these channels.

INTRODUCTION

Since A. F. Huxley suggested to Frankenhauser and Hodgkin that there is probably a localized potential on the surface of the cell membrane (Frankenhauser and...
Hodgkin, 1957), surface potential theory has been widely used to explain many phenomena involving electrostatic interactions in the membrane. The theory is significant because such a potential can establish an intramembrane electric field, which might then affect the function of many molecules residing in the membranes, particularly the gating mechanisms of voltage-dependent ion channels (McLaughlin, 1977; Green and Andersen, 1991). The consequences of the effect can be very important in terms of its influence on cellular functions such as excitability.

The chemical identity of the surface potential has been attributed to several different components in the membrane (Green and Andersen, 1991). (1) Membrane proteins contribute a significant amount of charges, carried by charged amino acids and other hydrophilic residues located at or near the aqueous surface of protein due to their hydrophilicity (Kyte and Doolittle, 1982; Catterall, 1988). (2) Charged lipids are also an important source contributing to the surface potential, especially phospholipids (Storch and Kleinfeld, 1985; Mato, 1990). At physiological pH, phospholipids exist as charged zwitterions, among which phosphatidylserine and phosphatidylinositol carry net negative charges. (3) Negatively charged sialic acid residues attached to asparagine-linked oligosaccharide chains (Kornfeld and Kornfeld, 1985) are normally added to the extracellular domain of membrane proteins during the glycosylation of membrane proteins (Catterall, 1988; Levinson, Thornhill, Duch, Recio-Pinto, and Urban, 1990). (4) Negatively charged phosphates can also be attached to membrane proteins following the phosphorylation of proteins by protein kinases, e.g., phosphorylation of the delayed rectifier K⁺ channels (Perozo, Bezanilla, and Dipolo, 1989).

The possible role of surface potential in the modulation of ion channel function has been extensively investigated. Various approaches have been used to alter this potential (for review, see Green and Andersen, 1991). (1) Changing ionic strength in the extracellular solution, particularly with divalent cations, is the classical way to modify (screen) surface potential. Following the leading work by Frankenhauser and Hodgkin (1957), many laboratories have observed the screening effects of divalent cations on the surface potential and the consequences on the behavior of different ion channels (McLaughlin, Szabo, and Eiseman, 1971; Hille, Woodhull, and Shapiro, 1975; Gilly and Armstrong, 1982; Hahin and Campbell, 1983; Armstrong and Matterson, 1986; Cukierman, Zinkand, French, and Krueger, 1988; Armstrong and Cota, 1990); (2) Changing pH is also an effective method to alter the surface charges (Campbell and Hahin, 1984; Zhang and Siegelbaum, 1991); (3) Various enzymes have been used as well in an attempt to modify the density of the surface charges; for example, the removal of sialic acid residues by neuraminidase (Yee, Weiss, and Langer, 1989; Levinson et al., 1990).

In this study, we have examined the effects of altering charge density on the surface membrane by introduction of charged lipid-like amphiphiles (Helenius, McCaslin, Fries, and Tanford, 1979) such as sodium dodecylsulfate (SDS) and dodecyltrimethylammonium (DDTMA). These amphiphiles can insert into the outer monolayer of the membrane, and, based on thermodynamic considerations, are unlikely to move through the hydrophobic center of the membrane to the inner monolayer over a short time period (several minutes), even if their movements are facilitated by a so-called endogenous “flip-pass.” This approach has been used
previously to examine the effects of these agents on the voltage-gated \( \text{Ca}^{2+} \) currents, contractile function, and \( \text{Ca}^{2+} \) uptake and binding in single cardiac myocytes (Burt and Langer, 1983; Philipson, Langer, and Rich, 1985; Langer and Rich, 1986; Post, Ji, Leonards, and Langer, 1991). After the exposure of myocytes to SDS and DDTMA, negatively charged amphiphile SDS increased \( \text{L-type Ca}^{2+} \) current (\( I_{\text{Ca,L}} \)), cell contraction, and \( \text{Ca}^{2+} \) uptake and binding, whereas positively charged amphiphile DDTMA had opposite effects (Post et al., 1991). The observed changes in both the amplitude and steady-state gating of the \( \text{L-type Ca}^{2+} \) current were in good agreement with the alterations of surface potential, assuming that the charged amphiphiles inserted into the outer monolayer of the sarcolemma (Ji, Weiss, and Langer, 1990; Post et al., 1991). These previous studies raised significant questions: (1) Are these effects specific to \( \text{L-type Ca}^{2+} \) channels? In other words, does the insertion also occur around other channels? If so, do other voltage-gated channels, e.g., Na\(^+\) and K\(^+\) channels, show similar alterations in terms of their voltage-dependent steady-state gating and macroscopic current? Can these alterations be interpreted as the results of modification of surface potential? (2) Besides the effects on the surface potential, is there a direct interaction between the charged amphiphiles and ion channels? Such an interaction may cause a conformational change of the channel protein with consequent changes of single channel properties, e.g., single channel conductance, contributing to the changes of macroscopic current. In this study, we further investigate the effects of these agents on voltage-dependent Na\(^+\) (\( I_{\text{Na}} \)) and delayed rectifier K\(^+\) current (\( I_{\text{K,del}} \)) in cardiac ventricular myocytes in order to address these questions and further characterize the effects of these amphiphiles.

Part of the work was reported previously in an abstract (Ji, Weiss, and Langer, 1992).

**MATERIALS AND METHODS**

**Isolation of Cardiac Ventricular Myocytes**

Adult rabbit or guinea pig ventricular myocytes were isolated by retrograde perfusion of the aortic artery with modified Tyrode's solution containing collagenase (class II; Worthington Biochemical Corp., Freehold, NJ) and protease or hyaluronidase (Sigma Immunochemicals, St. Louis, MO) (Mitra and Morad, 1985). The isolated rabbit ventricular myocytes were suspended in modified Eagle's medium (S-MEM; Irvine Scientific, Santa Ana, CA) and incubated overnight at 37°C to use on day 2. The guinea pig ventricular myocytes were used immediately after isolation.

**Whole-Cell Current Recording**

The patch-clamp technique was used to study the effects of the charged amphiphiles on voltage-dependent sodium and delayed rectifier potassium currents in the whole-cell recording configuration (Hamill, Marty, Neher, Sakmann, and Sigworth, 1981). The currents were recorded using an Axopatch 200 or Axopatch 1D patch-clamp amplifier (Axon Instruments, Inc., Foster City, CA) and digitized and stored in a PC computer interfaced to the amplifier through a 40-kHz Labmaster board (Scientific Solutions Inc., Solon, OH). The recording electrodes had a resistance of \( \sim 1-2 \text{ M\Omega} \) when filled with our pipette solutions (Table I). Standard procedures of capacitance and series resistance compensation were used (Hamill et
Programming of voltage-clamp commands and data acquisition and analysis were performed utilizing the pCLAMP software package (Axon Instruments, Inc.). The experimental chamber (0.5 ml) containing the myocytes was continuously perfused at a flow rate of 3–4 ml/min. All experiments were done at room temperature, 22°C.

Solutions

Pipette and extracellular solutions used for isolation of Na⁺ current (\(I_{\text{Na}}\)) and delayed rectifier K⁺ (\(I_{\text{K,del}}\)) are listed in Table I.

The charged amphiphiles we used were SDS and DDTMA (both from Sigma Immunochemicals). They are oppositely charged at the headgroup, negative for SDS but positive for DDTMA, and have an identical 12-carbon backbone. The concentration of the amphiphiles used was 20 μM, which was >40-fold below the critical micellar concentration of ~8.13 mM for SDS and ~14.6 mM for DDTMA. At this concentration, the detergents exist in the form of monomers (Helenius et al., 1979).

Data Analysis

All results in both figures and text are expressed as mean ± SEM.

RESULTS

Isolation of \(I_{\text{Na}}\)

The voltage-dependent \(I_{\text{Na}}\) was isolated by blocking \(I_{\text{Ca,L}}\) with 2 μM nifedipine and blocking K⁺ currents with tetraethylammonium (TEA) from the extracellular side and Cs⁺ from the intracellular side in overnight-incubated or freshly dissociated rabbit ventricular myocytes. To minimize the potential errors in the accuracy of clamp

### Table I

<table>
<thead>
<tr>
<th></th>
<th>(I_{\text{Na}})</th>
<th>(I_{\text{K,del}})</th>
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<tbody>
<tr>
<td></td>
<td>Internal</td>
<td>External</td>
</tr>
<tr>
<td>NaCl</td>
<td>5.0</td>
<td>5.3</td>
</tr>
<tr>
<td>NaOH</td>
<td>—</td>
<td>4.7</td>
</tr>
<tr>
<td>CsCl</td>
<td>138.5</td>
<td>—</td>
</tr>
<tr>
<td>CsOH</td>
<td>11.5</td>
<td>—</td>
</tr>
<tr>
<td>TEA-Cl</td>
<td>—</td>
<td>135.0</td>
</tr>
<tr>
<td>Choline-Cl</td>
<td>— (135.0)</td>
<td>—</td>
</tr>
<tr>
<td>K-glutamate</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>KCl</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>KOH</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>—</td>
<td>1.0</td>
</tr>
<tr>
<td>EGTA</td>
<td>5.0</td>
<td>—</td>
</tr>
<tr>
<td>HEPES</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.2</td>
<td>7.4</td>
</tr>
<tr>
<td>Glucose</td>
<td>—</td>
<td>10.0</td>
</tr>
<tr>
<td>MgATP</td>
<td>5.0</td>
<td>—</td>
</tr>
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</table>
pulses by series resistance, the magnitude of the macroscopic Na⁺ current was reduced by lowering extracellular [Na⁺] to 10 mM with substitution of choline, TEA, or N-methyl-D-glutamine and keeping intracellular [Na⁺] at 5 mM (Table I). The typical recording of the isolated INa under these conditions is shown in Fig. 1. The current was elicited by 55-ms test pulses to different membrane potentials (−70 to 30 mV) in 10-mV steps from a holding potential of −110 mV.

**Effects of the Charged Amphiphiles on INa**

The amplitude of INa was significantly affected by both charged amphiphiles (Fig. 2). As shown in Fig. 2 A, the maximal peak current increased by ~20% after exposure to SDS. From the plot of the peak Na⁺ current–voltage (I-V) relationships (Fig. 2 B), it was found that after exposure to SDS (1) the maximal peak INa was increased by ~20%; (2) INa was activated at a membrane potential 10 mV more negative (−60 mV as compared with −50 mV); (3) the maximal peak INa occurred at −40 mV instead of potential of −30 mV from a holding potential of −110 mV under control conditions and at a test membrane potential of −20 mV from the same holding potential after exposure to 20 μM DDTMA. (D) The I-V relations before and after 20 μM DDTMA superfusion.
−30 mV. In six cells, the maximal peak \( I_{Na} \) was increased by 19.6 ± 11.7% (\( n = 6; P < 0.05 \), paired \( t \) test) after exposure to 20 \( \mu \)M SDS. After SDS treatment, a holding potential of −120 mV was applied to maintain channels fully available for opening because of the possibility that the channels would be partially inactivated due to a shift of the steady-state inactivation of the current in the negative direction (see Fig. 3).

On the other hand, the exposure of myocytes to 20 \( \mu \)M DDTMA produced opposite effects (Fig. 2 C). The maximal peak current was decreased and there was a shift of the peak and the activation threshold of the current in the positive direction (Fig. 2 D). The decrease in peak \( I_{Na} \) in five cells averaged 18.1 ± 6.37% (\( P < 0.01 \), paired \( t \) test).

The reversal potential of \( I_{Na} \), however, was not significantly changed by either agent (Fig. 2, B and D).

**Effects of the Amphiphiles on the Steady-State Inactivation and Activation of \( I_{Na} \)**

As shown by the \( I-V \) relations (Fig. 2, B and D), the peak and the activation threshold of \( I_{Na} \) were shifted along the voltage axis by the amphiphiles, suggesting that the
voltage dependence of the steady-state inactivation and activation might be altered also. Fig. 3A shows the effects of SDS on the steady-state inactivation and activation of \( I_{\text{Na}} \). The steady-state inactivation was measured by recording the peak \( I_{\text{Na}} \) during a voltage clamp to \(-30\) mV after a 500-ms conditioning pulse to various membrane potentials ranging from \(-150\) to \(0\) mV and normalizing the currents to the maximum. The normalized peak currents were fit to a Boltzmann relationship:

\[
 h_{\text{inact}} = \frac{1}{1 + \exp\left[z\varepsilon (V - V_{1/2})/kT\right]}
\]

where \( z \) is the apparent valence of the inactivation gate, \( \varepsilon \) is the electric charge, \( k \) is the Boltzmann constant, \( T \) is the absolute temperature, \( V \) is the conditioning membrane potential, and \( V_{1/2} \) is the membrane potential corresponding to 50% inactivation. The inactivation curve (or \( h_{\text{inact}} \) curve, by Hodgkin and Huxley’s convention [Hodgkin and Huxley, 1952]) was shifted in the hyperpolarizing direction after exposure to SDS with \( V_{1/2} \) shifted by \(-22.1\) mV \((n = 7)\). However, the slope of the curve did not show a significant change (Fig. 3A).

The activation of \( I_{\text{Na}} \) was measured by clamping the membrane potential to various levels ranging from \(-70\) to \(0\) mV from a holding potential of \(-110\) mV. The whole-cell \( \text{Na}^+ \) channel conductance (\( g_{\text{Na}} \)) was calculated by dividing the peak \( I_{\text{Na}} \) by the driving force \((V_m - E_{\text{Na}})\) assuming that \( E_{\text{Na}} = 18\) mV for \([\text{Na}^+]_o = 10\) mM and \([\text{Na}^+]_i = 5\) mM. \( g_{\text{Na}} \) was normalized to the maximal value \( g_{\text{max}} \) and fit to a Boltzmann relationship:

\[
 g/g_{\text{max}} = \frac{1}{1 + \exp\left[z\varepsilon (V_{1/2} - V)/kT\right]}\]

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Table II

<table>
<thead>
<tr>
<th></th>
<th>Control ((n = 7))</th>
<th>(20\ \mu\text{M SDS} ) ((n = 7))</th>
<th>Control ((n = 5))</th>
<th>(20\ \mu\text{M DDTMA} ) ((n = 5))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Activation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( V_{1/2} ) (mV)</td>
<td>(-32.9 \pm 1.0)</td>
<td>(-41.1 \pm 1.3)</td>
<td>(-38.5 \pm 1.6)</td>
<td>(-28.4 \pm 1.6)</td>
</tr>
<tr>
<td>( z )</td>
<td>(3.9 \pm 0.3)</td>
<td>(3.8 \pm 0.4)</td>
<td>(4.1 \pm 0.2)</td>
<td>(3.7 \pm 0.4)</td>
</tr>
<tr>
<td><strong>Inactivation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( V_{1/2} ) (mV)</td>
<td>(-75.7 \pm 1.2)</td>
<td>(-97.8 \pm 1.5)</td>
<td>(-75.6 \pm 0.4)</td>
<td>(-71.5 \pm 0.9)</td>
</tr>
<tr>
<td>( z )</td>
<td>(2.9 \pm 0.3)</td>
<td>(3.0 \pm 0.3)</td>
<td>(2.8 \pm 0.4)</td>
<td>(2.7 \pm 0.3)</td>
</tr>
</tbody>
</table>

Conversely, positively charged DDTMA had opposite effects on the voltage dependence of \( I_{\text{Na}} \) inactivation and activation (Fig. 3B). \( V_{1/2} \) of the activation curve was shifted in the depolarizing direction by \(10.1\) mV \((n = 5)\). \( V_{1/2} \) of the steady-state
inactivation curve was shifted by 4.5 mV ($n = 5$) in the same direction. The averaged values of $V_{1/2}$'s and the calculated valences of the activation and inactivation gates before and after exposure of the cells to DDTMA are listed in Table II.

**No Significant Change of the Peak Whole-Cell Conductance**

As shown in Fig. 4, neither SDS nor DDTMA significantly altered the maximal whole-cell conductance, suggesting that the changes in the magnitude of $I_{Na}$ caused by the amphiphiles resulted from the shifts in the voltage dependence of the current gating and not from the direct effects on channel conductance.

![Figure 4. Effects of amphiphiles on the whole-cell Na$^+$ conductance. The whole-cell Na$^+$ channel conductance was calculated from the maximal peak $I_{Na}$ recorded at various membrane potentials by assuming a reversal potential of 18 mV. (A) The whole-cell Na$^+$ conductance before and after 20 μM SDS and (B) before and after 20 μM DDTMA. Note that the maximal conductance was not affected by either amphiphile.](image)

**No Open Channel Facilitation of the Effect of SDS on the Steady-State Inactivation**

To determine whether the ability of SDS to alter the steady-state inactivation of $I_{Na}$ was affected by the state of the Na$^+$ channel (closed, open, or inactivated), a train of depolarizing pulses was delivered before the conventional double-pulse protocol in order to drive Na$^+$ channels into the open or inactivated state for a greater percentage of time. Fig. 5 shows that the steady-state inactivation curve was not significantly altered by the train of depolarization pulses either before or after SDS treatment, suggesting that the effect of SDS was not dependent on the state of the Na$^+$ channel.
FIGURE 5. No open channel facilitation of the effects of SDS on the steady-state inactivation. Steady-state inactivation was examined using conventional double-pulse protocol (open symbols) and a double-pulse protocol preceded by a train of five depolarizing pulses to −30 mV for 100 ms from a holding potential of −120 mV delivered at 10 Hz (filled symbols). The protocol is shown in the upper panel. The $V_{1/2}$ and slope of the steady-state inactivation curves were not significantly altered by the train either before or after exposure to SDS.

FIGURE 6. The effects of the amphiphiles on the activation and inactivation kinetics of $I_{Na}$. (A) $I_{Na}$ normalized to the same peak amplitude before and after amphiphile treatment with SDS (upper traces) and DDTMA (lower traces). (B) The inactivation time constants ($\tau_{in}$) of $I_{Na}$ before (open symbols) and after (filled symbols) amphiphile treatment. $\tau_{in}$ was obtained by fitting the inactivating phase of $I_{Na}$ to a single exponential.
Effects of the Amphiphiles on the Activation and Inactivation Kinetics of \( I_{Na} \)

In addition to the effects on \( I_{Na} \) amplitude and steady-state activation and inactivation, the charged amphiphiles also altered the kinetics of \( I_{Na} \) activation and inactivation. When \( I_{Na} \) before and after exposure to the amphiphiles was scaled to the same peak amplitude, alterations in both the activation and inactivation phases of the currents were quite obvious (Fig. 6 A). Both activation and inactivation were accelerated by SDS and slowed by DDTMA. Activation was too rapid to fit accurately, but the inactivation phase was well fit to a single exponential, yielding the inactivation time constants (Fig. 6 B). The time constants (\( \tau_h \)) were decreased by SDS, but increased by DDTMA (Fig. 6 B). In both cases, \( \tau_h \) was shifted along the voltage axis in a parallel fashion. However, the magnitude of the shift along the voltage axis produced by either amphiphile was considerably greater than the corresponding shift in steady-state inactivation and activation (Fig. 3).

Concentration Dependence of the Effects on Steady-State Inactivation of \( I_{Na} \) by SDS

The effects of SDS on the steady-state inactivation of \( I_{Na} \) occurred in a concentration-dependent fashion. Fig. 7 A shows that the voltage dependence of the steady-state...
inactivation was shifted progressively in the negative direction as the concentration of SDS was increased from 20 to 100 μM. As shown in Fig. 7B, $V_{1/2}$ was shifted by $-21.1$ mV with 20 μM SDS, by $-32.0$ mV with 50 μM SDS, and by $-43.1$ mV with 100 μM SDS ($n = 6$).

**Screening the Effects of SDS on the Steady-State Inactivation by Increasing Extracellular [Ca$^{2+}$]**

Ca$^{2+}$ is one of the most widely used divalent cations to screen the negative surface charges, although it may also block sodium channel in a voltage-dependent manner (Yamamoto, Yeh, and Narahashi, 1984, 1985; Nillius, 1988). We therefore investigated whether the effects of SDS on $I_{Na}$ could be reversed by increasing extracellular [Ca$^{2+}$] to screen the negative charges of amphiphiles inserted in the outer monolayer of the membrane. After exposure to 20 μM SDS, the steady-state inactivation of $I_{Na}$ was shifted in the hyperpolarizing direction by $-22.1$ mV when extracellular [Ca$^{2+}$] was 1 mM. Increasing extracellular [Ca$^{2+}$] to 2.5 and 5.0 mM returned the inactivation curve toward the pre-SDS value by 6.1 and 17.0 mV, respectively (Fig. 8A; $n = 7$). A summary of $V_{1/2}$'s and the calculated valences of the inactivation gate under these conditions is shown in Table III. This effect of increasing extracellular [Ca$^{2+}$] was due to the screening of the inserted surface charges as well as the fixed,
Table III

Screening the Effects of SDS on Steady-State Inactivation of \( I_{Na} \) by Raising Extracellular [Ca\(^{2+}\)] (n = 7)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>20 ( \mu )M SDS</th>
<th>+2.5 mM Ca(^{2+})</th>
<th>+5.0 mM Ca(^{2+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_{1/2} ) (mV)</td>
<td>-75.7 ± 1.2</td>
<td>-97.8 ± 1.5</td>
<td>-91.8 ± 1.6</td>
<td>-80.7 ± 1.8</td>
</tr>
<tr>
<td>( z )</td>
<td>2.8 ± 0.3</td>
<td>3.0 ± 0.3</td>
<td>2.6 ± 0.2</td>
<td>2.5 ± 0.2</td>
</tr>
</tbody>
</table>

“native” negative surface charges, since in the presence of 5.0 mM Ca\(^{2+}\), 20 \( \mu \)M SDS produced a significantly smaller shift in \( V_{1/2} \) of the steady-state inactivation curve (\( h_{\infty} \) curve) (\( \Delta V_{1/2} = -7.6 \text{ mV}, n = 4 \)) (Fig. 8B) than in the presence of 1.0 mM Ca\(^{2+}\) (\( \Delta V_{1/2} = -22.1 \text{ mV} \)) (Fig. 8A). It should be noted that increasing [Ca\(^{2+}\)]\(_o\) caused progressive voltage-dependent block of \( I_{Na} \). In construction of the \( h_{\infty} \) curves, \( I_{Na} \) was therefore normalized to the maximal \( I_{Na} \) at each [Ca\(^{2+}\)]\(_o\), respectively. Since the magnitude of \( I_{Na} \) was elicited at a constant test potential, Ca\(^{2+}\)-dependent block of \( I_{Na} \) would be expected to have little effect on the intrinsic shape of the \( h_{\infty} \) curves at different [Ca\(^{2+}\)]\(_o\).

Effects of the Amphiphiles on the Activation of \( I_{K,del} \)

Guinea pig ventricular myocytes were used to examine the effects of the amphiphiles on the delayed rectifier potassium current (\( I_{K,del} \)) (Matsuura, Ebara, and Imoto, 1987; Balser, Bennett, and Roden, 1990), since \( I_{K,del} \) is larger in this species (Sanguinetti and Jurkiewicz, 1990). \( I_{K,del} \) was isolated by blocking \( I_{Na} \) with 1 \( \times \) 10\(^{-5}\) M tetrodotoxin and \( I_{Ca,L} \) with 2 \( \times \) 10\(^{-6}\) M nifedipine (Fig. 9A). \( I_{K,del} \), elicited by voltage pulses to positive potentials from a holding potential of -30 mV, activated very slowly and was not fully activated at the end of a 7.2-s pulse. Tail currents were recorded after

Figure 9. Recording of the delayed rectifier potassium current. (A) The delayed rectifier potassium current elicited by depolarizing the myocytes to various membrane potentials (as indicated) positive to the holding potential of -30 mV in guinea pig ventricular myocytes. (B) Tail currents of the delayed rectifier potassium current recorded when the myocytes were clamped back to -30 mV.
clamping the myocytes back to a holding potential of \(-30\) mV (Fig. 9 B) and plotted against the test potentials to obtain the activation curve of \(I_{K,\text{del}}\) (Fig. 10).

After exposing myocytes to the amphiphiles, the activation curves of \(I_{K,\text{del}}\) were shifted in the negative direction by SDS and in the positive direction by DDTMA (Fig. 10, A and B), similar to the shifts of \(I_{Na}\). The shifts in the \(V_{1/2}\) were \(-7.1 \pm 1.7\) \((n = 7)\) for SDS and \(10.2 \pm 3.0\) \((n = 4)\) for DDTMA, respectively. Without amphiphile treatment, no significant shift in \(V_{1/2}\) was observed over a comparable time period after the control recording (Fig. 10 C).

**Figure 10.** The effects of the amphiphiles on the normalized tail currents of the delayed rectifier potassium current. The tail currents were normalized to the maximal value and plotted against the test potentials. Open symbols (○) are the recordings under control conditions and solid symbols (■) are the experimental data points 3 min after exposure to either 20 μM SDS (A), 20 μM DDTMA (B), or no amphiphile (C). The solid lines are the best fits to the Boltzmann relation.

Under control conditions, \(I_{K,\text{del}}\) showed a notable rundown after breaking into the cell (Fig. 11 A), which is believed to be due to inadequate phosphorylation of channel proteins (Giles, Nakajima, Ono, and Shibata, 1989). However, the rundown of the current was not obviously altered by either amphiphile (Fig. 11, B and C). Consistent with the shifts in the voltage dependence of current activation, SDS increased and...
DDTMA decreased the magnitude of $I_{K,\text{tail}}$ over the membrane potential range from $-20$ to $20$ mV.

**DISCUSSION**

The importance of the surface potential in the modulation of voltage-dependent ionic currents has been long recognized (Frankenhauser and Hodgkin, 1957; Hille, 1992). In our previous studies (Ji et al., 1990; Post et al., 1991), we found that the steady-state kinetics of voltage-gated L-type Ca$^{2+}$ current, and consequently the amplitude of the current, were altered by charged amphiphiles. We hypothesized that the changes in the steady-state gating were probably the consequence of the modification of surface potential by the charged amphiphiles inserting into the outer monolayer of the cell membrane. In this study, we have found that the charged amphiphiles also altered the amplitude and the voltage dependence of steady-state gating of both voltage-gated Na$^+$ and delayed rectifier K$^+$ currents in a manner
consistent with altered surface potential. This study therefore supports the idea that
insertion of the amphiphiles occurs in the lipid environment surrounding Na\(^+\) and
K\(^+\) channels, and is not just specific to the L-type Ca\(^{2+}\) channels. At this point,
however, it cannot be concluded whether the insertion occurs in an evenly smeared or
an uneven discrete manner. Finally, our study suggests that direct channel protein–
amphiphile interactions are also likely to be present.

**Effects of the Amphiphiles on \(I_{\text{Na}}\) and Surface Potential**

Similar to the effects on \(I_{\text{Ca,L}}\) (Ji et al., 1990; Post et al., 1991), the maximal peak \(I_{\text{Na}}\) was increased after superfusion of myocytes with negatively charged SDS, but
decreased with positively charged DDTMA. These changes in the amplitude of the
maximal peak current could be adequately explained by the shifts of the voltage
dependence of current activation along the voltage axis (Fig. 3). In the case of SDS, a
shift in the negative direction of the \(I_{\text{Na}}\) activation curve caused a larger fraction of
channels to open at an equivalent membrane potential, increasing the whole-cell Na\(^+\)
conductance at that membrane potential. As a consequence, the macroscopic current
increased, according to the relation: \(I = G \times (V_{\text{m}} - E_{\text{ion}})\), in which \(G\) is the whole-cell
conductance (product of \(N\) [number of total functioning channels], \(\gamma\) [single channel
conductance], and \(P_o\) [open probability of channels, or fraction of open channels]),
\(V_{\text{m}}\) is the membrane potential, and \(E_{\text{ion}}\) is the reversal potential for the ion. The
positively charged DDTMA, on the other hand, shifted the activation curve in the
depolarizing direction and therefore produced an opposite effect: decrease of
macroscopic current due to a decrease of \(P_o\). The possibility that the amphiphiles
affected the magnitude of macroscopic \(I_{\text{Na}}\) by altering \(\gamma\) and \(N\) is unlikely since the
maximal whole-cell conductance was similar before and after exposure to SDS or
DDTMA (Fig. 4). This is consistent with the observation that the \(I-V\) plots before and
after amphiphile treatment were closely superimposed once the current was fully
activated above 0 mV (Fig. 2). The estimation that the extracellular mouth of the Na\(^+\)
channel may protrude well beyond the diffuse double layer (Green, Weiss, and
Andersen, 1987) would also make it unlikely that changes of [Na\(^+\)] in the diffuse
double layer after the insertion of the amphiphiles might affect \(\gamma\).

The shifts of the activation and inactivation of \(I_{\text{Na}}\) can be explained by a change of
surface potential due to the intercalation of the charged amphiphiles into the outer
monolayer of the sarcolemma. Under physiologic conditions, fixed negative surface
charges at the aqueous faces of the membrane, carried by integral membrane
proteins, sugar moieties, and phospholipids (see Introduction) are believed to
produce local potentials (surface potentials) which construct an intramembrane field,
electrostatically affecting the operation of voltage-sensitive membrane proteins. This
is described by the following relation:

\[
V_{\text{eff}} = V_{\text{m}} + (\psi_i - \psi_o)
\]

in which \(V_{\text{eff}}\) is the overall effective potential across the membrane, \(V_{\text{m}}\) is the
membrane potential measured in the bulk solutions inside and outside of the
sarcolemma, and \(\psi_i\) and \(\psi_o\) are the surface potentials at the inner and outer surfaces
of the membrane. After intercalation into the outer monolayer, negatively charged
SDS should increase the negativity of \(\psi_o\) and reduce the difference between \(\psi_o\) and \(\psi_i\),
causing a partial depolarization of $V_{\text{eff}}$ and consequently shifting the steady-state inactivation and activation in the hyperpolarizing direction. Positively charged DDTMA, on the other hand, is expected to produce opposite effects: a decrease in the negativity of $\psi_0$ causing a partial hyperpolarization of $V_{\text{eff}}$ and therefore a shift of the activation and inactivation curves in the depolarizing direction.

The effects of SDS on steady-state inactivation of $I_{\text{Na}}$ were concentration dependent. This is consistent with the Gouy-Chapman-Stern surface potential theory (McLaughlin, 1977; Cevc, 1990), which predicts that the concentration of anions at the membrane surface ([$A^-]_x = 0$) is a function of the concentration of anionic amphiphiles in the bulk solution ([$A^-]_x = \infty$), before reaching the maximum capacity of insertion. According to the theory, the surface charge density is related to surface potential by the relation (Graham, 1947):

$$\sigma^2 = 2\varepsilon_0 \varepsilon R \sum c_i \left[ \exp\left(-z_i F \varphi / RT \right) - 1 \right]$$

where $\sigma$ is the surface charge density, $\varphi$ is the surface potential, $\varepsilon$ is the dielectric constant for aqueous solution, $\varepsilon_0$ is the permittivity of free space, $c_i$ is the concentration of the $i$th ion in the bulk solution, and $z_i$ is the valence of the $i$th ion. When an anionic amphiphile is added, $\sigma$ will be changed after the absorption of the anion to the membrane according to:

$$\sigma' = \frac{1}{K}(\sigma'_{\text{max}} - \sigma') [A^-]_{x = 0}$$

where $\sigma'$ is the charge density of the amphiphiles absorbed per unit area, $\sigma'_{\text{max}}$ is the maximum absorption, $K$ is the dissociation constant, and $[A^-]_{x = 0}$ is a function of $[A^-]_{x = \infty}$ expressed via the Boltzmann relationship:

$$[A^-]_{x = 0} = [A^-]_{x = \infty} \exp(\varphi_0 F / RT)$$

It is clear from Eqs. 5 and 6 that the total density of surface charges ($\sigma + \sigma'$), and therefore the magnitude of surface potential, is dependent on the $[A^-]_{x = \infty}$. In our case, $V_{1/2}$ of the steady-state inactivation of $I_{\text{Na}}$ was progressively shifted in the negative direction as the concentration of SDS was increased from 20 to 100 $\mu$M, consistent with a continuous absorption of anionic amphiphiles over this concentration range.

The ability of increased extracellular $[Ca^{2+}]$ to partially reverse the shift in $V_{1/2}$ of the steady-state inactivation of $I_{\text{Na}}$ by SDS is also consistent with the idea that the effects of SDS on $I_{\text{Na}}$ are mediated via changes in surface potential (Fig. 8A) since it is well known that $Ca^{2+}$ screens negative surface charges (Hille et al., 1975). The screening effects of $Ca^{2+}$ on the inserted anions (and the fixed surface charges as well) may only be fractional. This is due to the insertion of more SDS when $\varphi_0$ is reduced by the screening effects of $Ca^{2+}$ since the insertion of SDS is also a function of $\varphi_0$ (Eq. 6). However, in Gouy-Chapman-Stern theory, the lipophilicity of negatively charged amphiphiles was not considered. The insertion of anionic amphiphiles may be dependent on this property as well as on $\varphi_0$. This seems to be the case in this study because: (1) the shift of $I_{\text{Na}}$ inactivation curve by SDS showed strong concentration dependence despite an increase of $\varphi_0$ in the negative direction resulted from the continuous insertion of SDS (Fig. 7); and (2) the shift was at least partially removed.
by raising extracellular $[\text{Ca}^{2+}]$ despite a decrease in the negativity of $\psi$, due to the screening effect of $\text{Ca}^{2+}$, which would be expected to facilitate SDS insertion.

Besides the effects on the amplitude and steady-state gating of $I_{\text{Na}}$, the activation and inactivation kinetics of the current were also changed by the amphiphiles. Currents recorded at the same testing potential showed that both activation and inactivation were accelerated by SDS but slowed by DDTMA (Fig. 6 A). $\tau_i$ was shifted in the hyperpolarizing direction by SDS but in the depolarizing direction by DDTMA (Fig. 6 B). The changes of both activation and inactivation kinetics of $I_{\text{Na}}$ are consistent with the result of alterations of surface potential following amphiphile treatment. However, as shown by Fig. 6 B, the magnitude of shift in the time constants of inactivation was much greater than the shifts in the steady-state activation and inactivation. It is, therefore, tempting to speculate about the possibility that additional effects by the amphiphiles on the inactivation mechanism of the sodium channels were present (see below).

**Asymmetry of the Shifts by SDS and DDTMA**

Although shifts of steady-state activation and inactivation of $I_{\text{Na}}$ can be attributed to surface potential alterations by the amphiphiles, the asymmetry between the effects of two compounds is inconsistent with the assumption that the amphiphiles exert their effects exclusively by modifying surface potential. In our previous work, both the steady-state activation and inactivation of $I_{\text{Ca,L}}$ were similarly shifted by SDS and DDTMA by $\sim 10$ mV (Ji et al., 1990; Post et al., 1991). This study shows that the shift in $V_{1/2}$ of the $I_{\text{Na}}$ activation curve ($\sim 10$ mV) was less pronounced than that of the steady-state inactivation ($\sim 20$ mV) or the time constants of the current inactivation ($\sim 40$ mV; Figs. 3 A and 6 B) in the case of SDS, but more pronounced in the case of DDTMA. Similar asymmetrical shifts in $I_{\text{Na}}$ activation and inactivation were also observed previously in several studies involving modification of surface potential by different agents (La$^{3+}$, Armstrong and Cota, 1990; trinitrobenzene sulfonic acid, Cahalan and Pappone, 1981; Ca$^{2+}$, Frankenhauser and Hodgkin, 1957; Zn$^{2+}$, Gilly and Armstrong, 1982), and may suggest that the electrical field sensed by the protein is very complex. One could also argue that these phenomena may reflect a different sensitivity of steady-state activation and inactivation of $I_{\text{Na}}$ to surface potential alteration. However, this seems inconsistent with the substantial evidence that $I_{\text{Na}}$ inactivation is not intrinsically voltage dependent, and that its apparent voltage dependence is related to the voltage dependence of $I_{\text{Na}}$ activation (Armstrong and Bezanilla, 1977; Armstrong, 1981; Cota and Armstrong, 1989). Thus, to explain this asymmetry, it seems likely that the charged amphiphiles must interact directly with the charges borne by channel proteins, possibly via a mechanism involving electrostatic attraction or repulsion. One possibility is that the amphiphiles interact with fatty acids covalently linked to the Na channel protein. It is conceivable that the portion of the channel protein with which SDS interacts to influence the voltage dependence of steady-state inactivation is more positively charged than the site at which it interacts to influence activation. Then, because of electrostatic interaction, negatively charged SDS might accumulate to a greater extent at the site influencing inactivation than at the site influencing activation, causing a greater shift in the
voltage dependence of inactivation than activation. Since DDTMA is positively charged, it would be expected to have opposite effects, as was observed experimentally (Fig. 3). This hypothesis is consistent with the notion that activation and inactivation gates of voltage-dependent ion channels contain charged amino acid residues, such as lysines and arginines located in the S4 segment and the intracellular loop between domains 3 and 4 of the channel primary structure (Barchi, 1988; Catterall, 1988; Stühmer, Conti, Suzuki, Wang, Noda, Yahagi, Kubo, and Numa, 1989). Whether the amphiphiles could interact with the inactivation mechanism directly through the hydrophilic, pore-forming portion of the channel when it is open, or through the lipoprotein interface is speculative. It seems unlikely that the charged amphiphiles may gain access to the sodium channel when the voltage sensors are exposed to extracellular side during depolarization (Sammar, Spira, and Meiri, 1992) since the effects on the steady-state inactivation were not facilitated by a depolarizing train (Fig. 5).

Effects on the Delayed Rectifier K+ Current
Effects of amphiphiles on the delayed rectifier K+ current were studied in single ventricular myocytes from guinea pig since $I_{K,del}$ is generally larger in this species than in rabbit ventricular myocytes (Sanguinetti and Jurkiewicz, 1990). Due to the slow activation and a rapid rundown of the current after the rupture of patch membrane, it was difficult to precisely quantitate the effects of amphiphiles on the current. However, as shown in Fig. 11, neither SDS nor DDTMA seemed to alter the rundown of $I_{K,del}$. Despite this, $I_{K,del}$ was increased by SDS and decreased by DDTMA in a voltage-dependent manner over the range of membrane potentials from −20 to 20 mV. The changes of $I_{K,del}$ over this membrane potential range could be explained by the shift of $I_{K,del}$ activation along the voltage axis, in the hyperpolarizing direction in the case of SDS and in the depolarizing direction in the case of DDTMA, similar to the effects on $I_{Na}$ activation produced by the amphiphiles. This is consistent with our hypothesis of an alteration of surface potential due to the insertion of charged amphiphiles around ion channels.

In summary, our observations demonstrate that the effects of the charged amphiphiles on the amplitude of $I_{Na}$ and $I_{K,del}$ can be largely explained by the alterations in their voltage-dependent steady-state gating due to a modification of surface potential after the insertion of amphiphiles into the outer monolayer of the membrane in the myocytes, which is consistent with the notion that the voltage-dependent gating mechanisms of these channels are located within the electrical field established by surface potential. It is therefore indicated, although indirectly, that the charges carried by membrane lipids are of significance in the modulation of channel gating. This agrees well with experiments in lipid planar bilayers, which have shown that channel gating mechanisms, unlike ion permeation properties, are influenced by lipid charges (Green et al., 1987; Cukierman et al., 1988). These charged amphiphiles may be useful as a tool to modify the density of surface charges in order to observe the interaction between surface charges and channel proteins. Besides the effects on surface potential, however, in the case of $I_{Na}$, a direct electrostatic interaction between the amphiphiles and channel gating mechanisms appears highly likely. Finally, the marked effects of these amphiphiles on cardiac excitation–
contraction coupling (Post et al., 1991) demonstrate that modification of surface charge can markedly influence physiological function. It is intriguing to speculate that alterations of surface potential under pathophysiological conditions, such as cardiac ischemia, in which dramatic changes in membrane phospholipid content occur, may contribute to altered cardiac function in these settings.

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