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 dodecyltrimethylammonium (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

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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 Eisenman, 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 Ca^{2+} currents, contractile function, and 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 L-type Ca^{2+} current ($I_{Ca,L}$), cell contraction, and 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 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 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_{Na}) and delayed rectifier K^+ current ($I_{K,del}$) 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 ~1–2 MΩ when filled with our pipette solutions (Table I). Standard procedures of capacitance and series resistance compensation were used (Hamill et

al., 1981; Cota and Armstrong, 1989). 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_{Na}) and delayed rectifier K^+ ($I_{K,del}$) 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).

TABLE I

Solutions for Isolation of I_{Na} and $I_{K,del}$

	I_{Na}		$I_{K,del}$	
	Internal	External	Internal	External
NaCl	5.0	5.3	—	—
NaOH	—	4.7	—	—
CsCl	133.5	—	—	—
CsOH	11.5	—	—	—
TEA-Cl	—	135.0	—	—
Choline-Cl	—	(135.0)	—	140.0
K-glutamate	—	—	108.2	—
KCl	—	—	20.0	0.6
KOH	—	—	16.8	4.8
$MgCl_2$	1.0	1.0	1.0	1.0
$CaCl_2$	—	1.0	—	1.0
EGTA	5.0	—	5.0	—
HEPES	10.0	10.0	10.0	10.0
pH	7.2	7.4	7.2	7.4
Glucose	—	10.0	—	10.0
MgATP	5.0	—	5.0	—

Data Analysis

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

RESULTS

Isolation of I_{Na}

The voltage-dependent I_{Na} was isolated by blocking $I_{Ca,L}$ 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

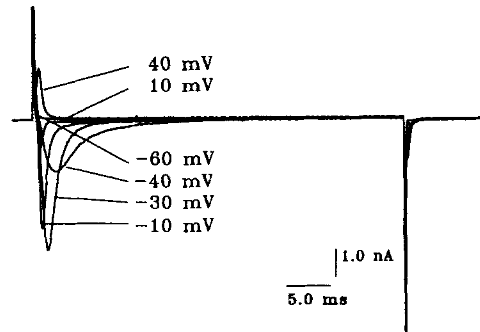

FIGURE 1. Isolation of I_{Na}. I_{Na} was recorded in the whole-cell recording configuration using the patch-clamp technique. Amplitude of the current was reduced by lowering extracellular [Na^+] to 10 mM, with substitution of Na^+ with choline or TEA. The intracellular [Na^+] was 5 mM. The holding potential was −110 mV.

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 I_{Na} 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 I_{Na}

The amplitude of I_{Na} 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 I_{Na} was increased by ~20%; (*2*) I_{Na} was activated at a membrane potential 10 mV more negative (−60 mV as compared with −50 mV); (*3*) the maximal peak I_{Na} occurred at −40 mV instead of

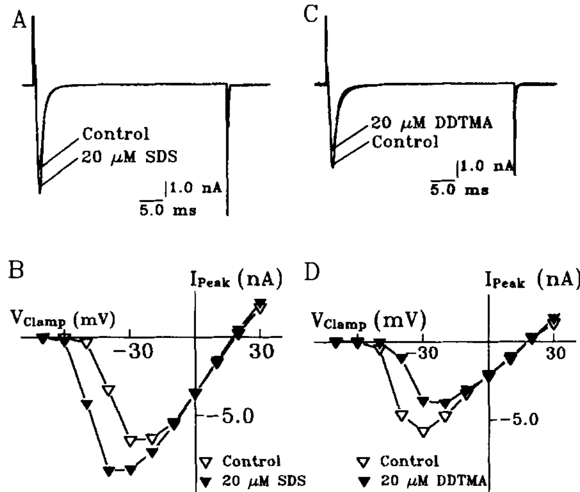

FIGURE 2. Effects of the charged amphiphiles on I_{Na}. (*A*) The effects of negatively charged SDS on the maximal peak I_{Na}. The current was elicited at a test membrane potential of −30 mV from a holding potential of −110 mV under control conditions and at a test membrane potential of −40 mV from a holding potential of −120 mV after exposure to 20 μM SDS. (*B*) The *I-V* plots before and after SDS treatment. (*C*) The effects of positively charged DDTMA on the maximal peak I_{Na}. The current was recorded at a test membrane 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 μM 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 μM 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

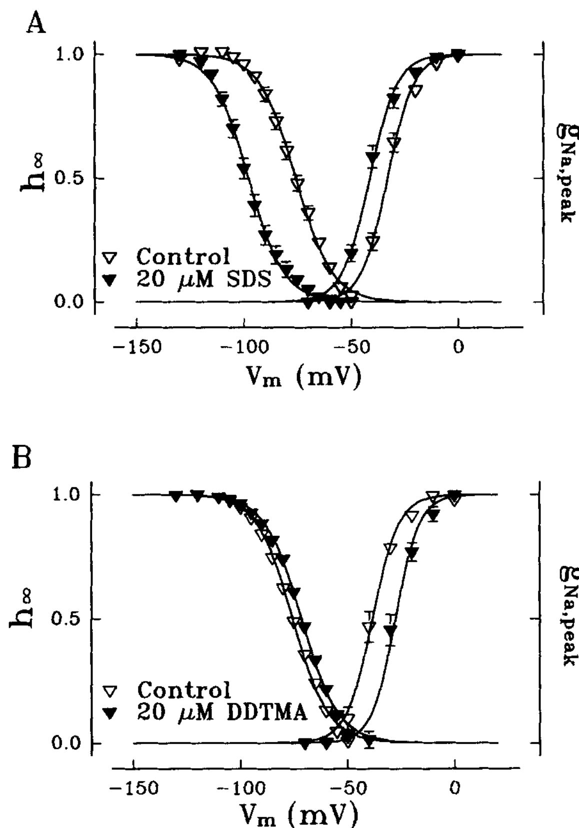

FIGURE 3. Effects of the charged amphiphiles on the steady-state activation and inactivation of I_{Na}. Open symbols represent the controls and filled symbols are experimental data points after treatment with SDS or DDTMA. Experimental data points are expressed as mean ± SEM. The solid lines are the best fits to the Boltzmann relation (see Results). (*A*) The effects of 20 μM SDS ($n = 7$). (*B*) The effects of 20 μM DDTMA ($n = 5$).

(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. 3 *A* shows the effects of SDS on the steady-state inactivation and activation of I_{Na}. The steady-state inactivation was measured by recording the peak I_{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:

$$h_\infty = 1/\{1 + \exp[ze(V - V_{1/2})/kT)]\} \tag{1}$$

where z is the apparent valence of the inactivation gate, e 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_∞ 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. 3 *A*).

TABLE II

Effects of the Charged Amphiphiles on $V_{1/2}$'s and the Apparent Valences of Steady-State Inactivation and Activation of I_{Na}

	Control (n = 7)	20 μM SDS (n = 7)	Control (n = 5)	20 μM DDTMA (n = 5)
Activation				
$V_{1/2}$ (mV)	−32.9 ± 1.0	−41.1 ± 1.3	−38.5 ± 1.6	−28.4 ± 1.6
z	3.9 ± 0.3	3.8 ± 0.4	4.1 ± 0.2	3.7 ± 0.4
Inactivation				
$V_{1/2}$ (mV)	−75.7 ± 1.2	−97.8 ± 1.5	−75.6 ± 0.4	−71.5 ± 0.9
z	2.9 ± 0.3	3.0 ± 0.3	2.8 ± 0.4	2.7 ± 0.3

The activation of I_{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 Na^+ channel conductance (g_{Na}) was calculated by dividing the peak I_{Na} by the driving force ($V_m - E_{Na}$) assuming that E_{Na} = 18 mV for $[Na^+]_o$ = 10 mM and $[Na^+]_i$ = 5 mM. g_{Na} was normalized to the maximal value (g_{max}) and fit to a Boltzmann relationship:

$$g/g_{max} = 1/\{1 + \exp[ze(V_{1/2} - V)/kT]\} \tag{2}$$

to obtain the 50% activation point and the valence of the activation gate. $V_{1/2}$ of the activation was shifted in the hyperpolarizing direction by −8.2 mV (n = 7), which was less than the shift in the steady-state inactivation caused by SDS (Fig. 3 *A* and Table II).

Mean values of $V_{1/2}$'s and the calculated apparent valences of the activation and inactivation gates from individual cells before and after exposure of the myocytes to SDS are summarized in Table II.

Conversely, positively charged DDTMA had opposite effects on the voltage dependence of I_{Na} inactivation and activation (Fig. 3 *B*). $V_{1/2}$ 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.

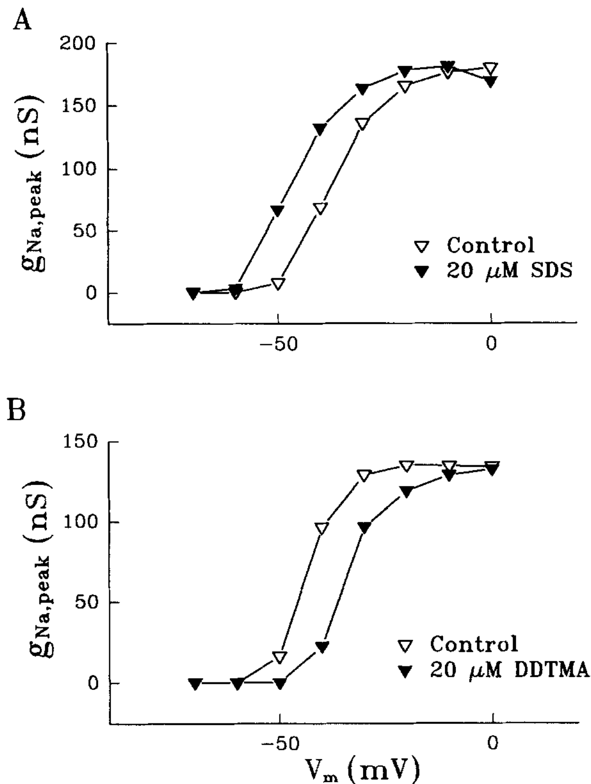

FIGURE 4. Effects of amphiphiles on the whole-cell Na^+ conductance. The whole-cell Na^+ channel conductance was calculated from the maximal peak I_{Na} recorded at various membrane potentials by assuming a reversal potential of 18 mV. (*A*) The whole-cell Na^+ conductance before and after 20 μM SDS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

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.

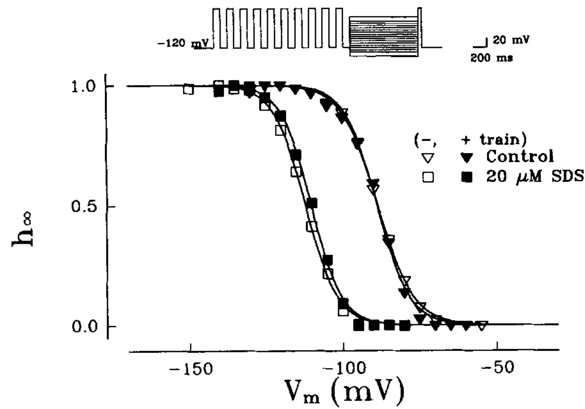

FIGURE 5. No open channel facilitation of the effects of SDS on the steady-state inactivation. Steady-state inactivation was examined using conventional double-pulse protocol (*open symbols*) and a double-pulse protocol preceded by a train of five depolarizing pulses to −30 mV for 100 ms from a holding 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.

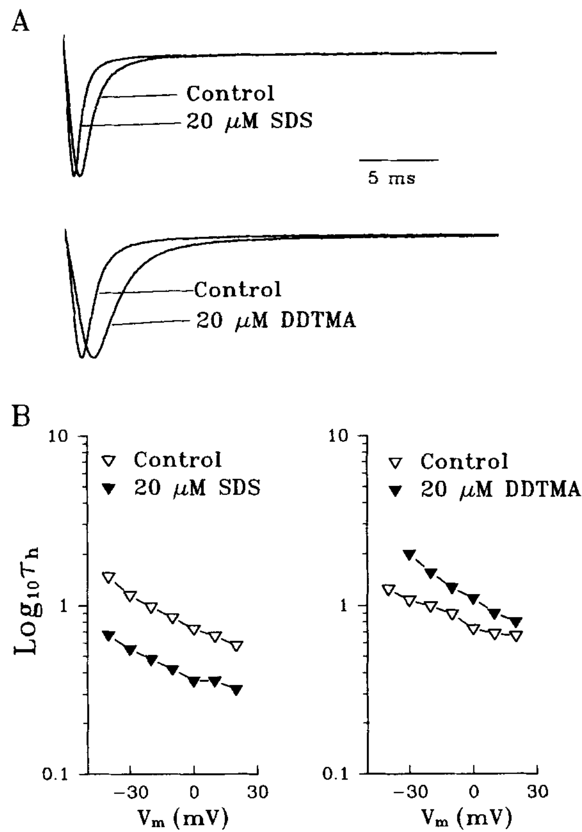

FIGURE 6. The effects of the amphiphiles on the activation and inactivation kinetics of I_{Na}. (*A*) I_{Na} normalized to the same peak amplitude before and after amphiphile treatment with SDS (*upper traces*) and DDTMA (*lower traces*). (*B*) The inactivation time constants (τ_h) of I_{Na} before (*open symbols*) and after (*filled symbols*) amphiphile treatment. τ_h 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

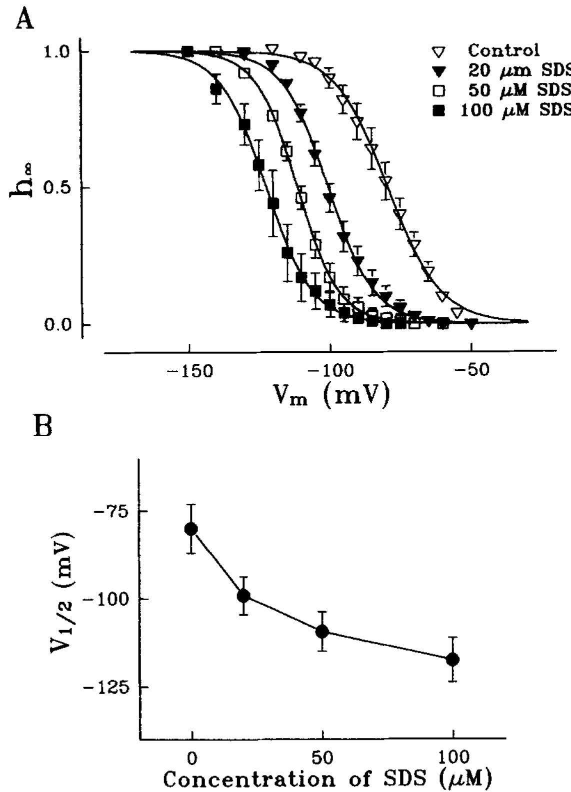

FIGURE 7. Concentration dependence of the effects of SDS on the steady-state inactivation of I_{Na}. (*A*) After recording the control curve (▽), [SDS] was raised from 20 μM (▼) to 50 μM (□) and 100 μM (■). The solid lines are the best fits to the Boltzmann relation. $V_{1/2}$ was progressively shifted in the negative direction as the concentration of SDS was increased. (*B*) Summary of $V_{1/2}$'s at different SDS concentrations ($n = 6$).

constants (Fig. 6 *B*). The time constants (τ_h) were decreased by SDS, but increased by DDTMA (Fig. 6 *B*). In both cases, τ_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. 7 *B*, $V_{1/2}$ 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

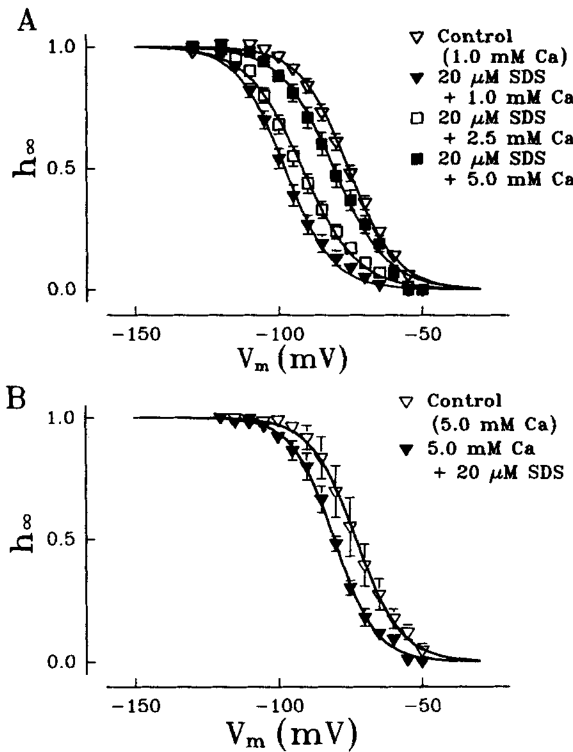

FIGURE 8. (*A*) Partial reversal of the effects of SDS on the steady-state inactivation of I_{Na} by raising extracellular [Ca^{2+}] from 1.0 mM (▼) to 2.5 mM (□) and 5.0 mM (■) in the presence of 20 μM SDS. The control curve before exposure to SDS is also shown (▽). The solid lines are the best fits to the Boltzmann relation (n = 7). (*B*) Dependence of SDS insertion on extracellular [Ca^{2+}]. The shift of the steady-state inactivation curve by SDS was less in the presence of 5.0 mM Ca^{2+} in the perfusate (n = 4) than in the presence of 1.0 mM Ca^{2+} (compare with *A*).

(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. 8 *A*; 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)

	Control	20 μM SDS	+2.5 mM Ca^{2+}	+5.0 mM Ca^{2+}
$V_{1/2}$ (mV)	−75.7 ± 1.2	−97.8 ± 1.5	−91.8 ± 1.6	−80.7 ± 1.8
z	2.8 ± 0.3	3.0 ± 0.3	2.6 ± 0.2	2.5 ± 0.2

"native" negative surface charges, since in the presence of 5.0 mM Ca^{2+}, 20 μM SDS produced a significantly smaller shift in $V_{1/2}$ of the steady-state inactivation curve (h_∞ curve) ($\Delta V_{1/2} = -7.6$ mV, $n = 4$) (Fig. 8 *B*) than in the presence of 1.0 mM Ca^{2+} ($\Delta V_{1/2} = -22.1$ mV) (Fig. 8 *A*). It should be noted that increasing $[Ca^{2+}]_o$ caused progressive voltage-dependent block of I_{Na}. In construction of the h_∞ 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_∞ 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, Ehara, 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 1×10^{-5} M tetrodotoxin and $I_{Ca,L}$ with 2×10^{-6} M nifedipine (Fig. 9 *A*). $I_{K,del}$, 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

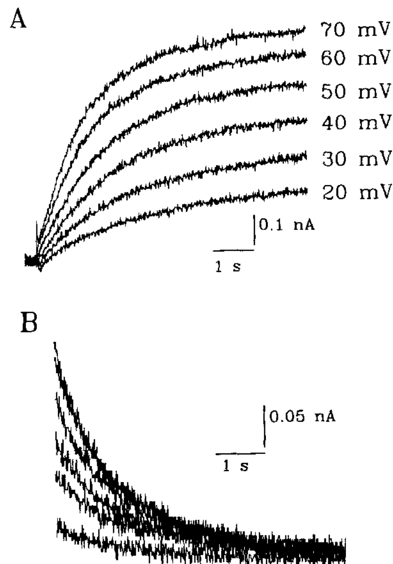

FIGURE 9. Recording of the delayed rectifier potassium current. (*A*) The delayed rectifier potassium current elicited by depolarizing the myocytes to various membrane potentials (as indicated) positive to the holding potential of −30 mV in guinea pig ventricular myocytes. (*B*) Tail currents of the 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 $I_{K,del}$ (Fig. 10).

After exposing myocytes to the amphiphiles, the activation curves of $I_{K,del}$ 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 ± 1.7 (n = 7) for SDS and 10.2 ± 3.0 (n = 4) for DDTMA, respectively. Without amphi-

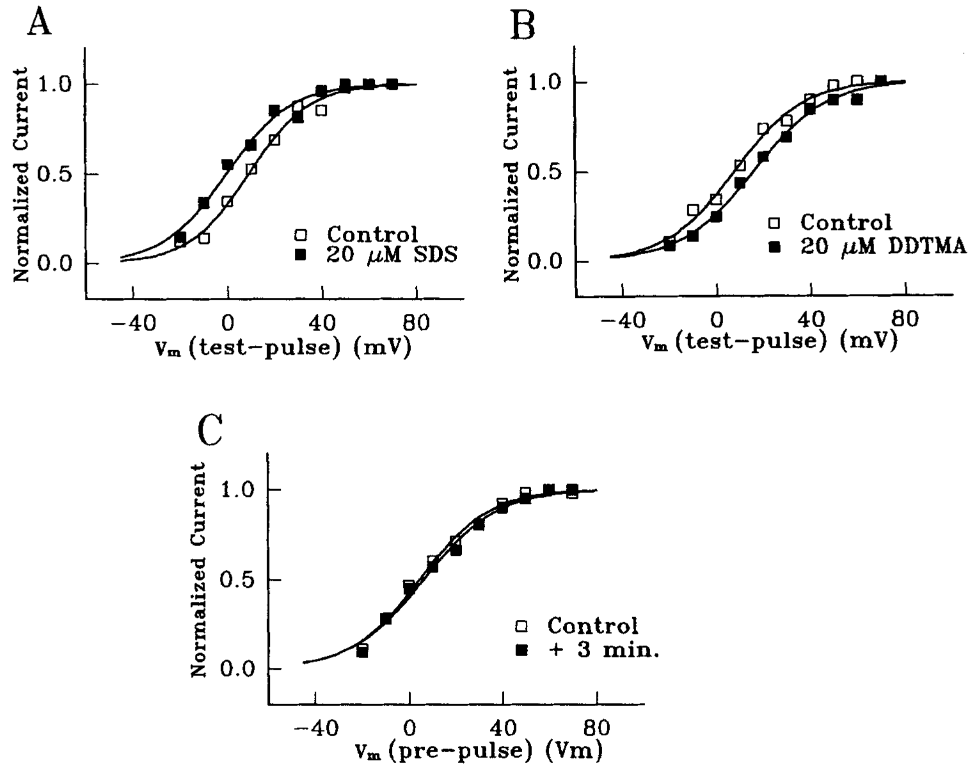

FIGURE 10. The effects of the amphiphiles on the normalized tail currents of the delayed rectifier potassium current. The tail currents were normalized to the maximal value and plotted against the test potentials. Open symbols (□) are the recordings under control conditions and solid symbols (■) are 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.

phile treatment, no significant shift in $V_{1/2}$ was observed over a comparable time period after the control recording (Fig. 10 *C*). Fig. 11 shows the *I-V* relations of $I_{K,del}$. Under control conditions, $I_{K,del}$ 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

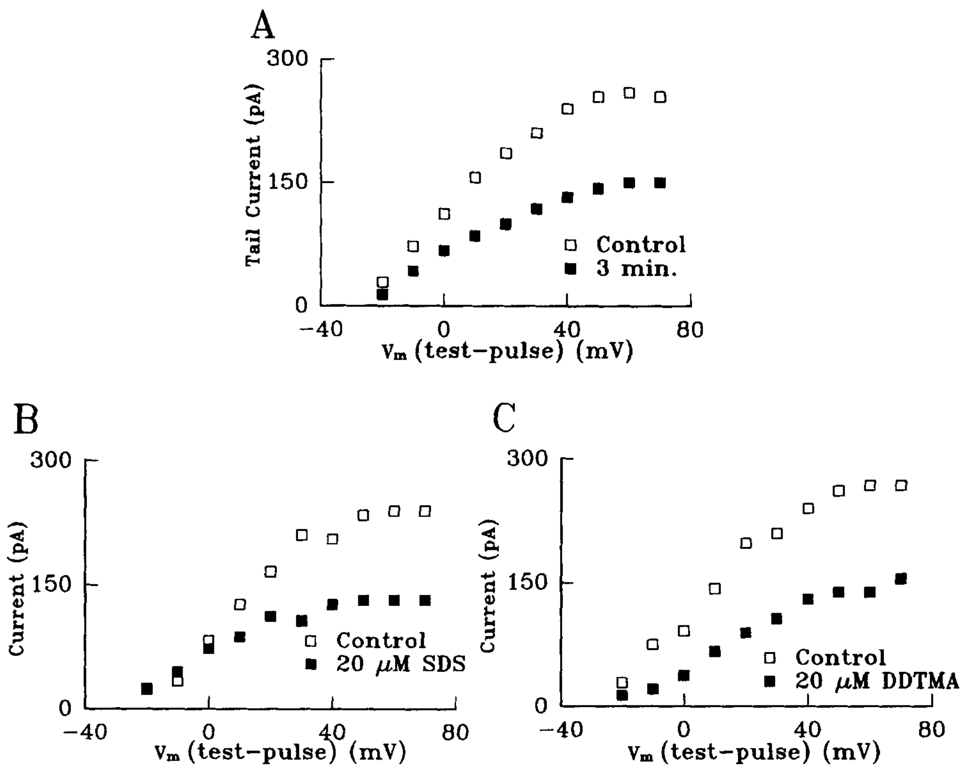

FIGURE 11. Effects of the amphiphiles on the magnitude of the tail currents of the delayed rectifier potassium current. The tail currents were measured arbitrarily at 6.9 ms after the end of the test pulse when the myocyte was clamped back to −30 mV and plotted against the test potentials. Open symbols (□) are the recordings under initial control conditions and solid symbols (■) are the recordings 3 min later under the same control condition (*A*) or with exposure to 20 μM SDS (*B*) or 20 μM DDTMA (*C*).

DDTMA decreased the magnitude of $I_{K,tail}$ 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_{Na} and Surface Potential

Similar to the effects on $I_{Ca,L}$ (Ji et al., 1990; Post et al., 1991), the maximal peak I_{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_{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: $I = G \times (V_m - E_{ion})$, in which G is the whole-cell conductance (product of N [number of total functioning channels], γ [single channel conductance], and P_o [open probability of channels, or fraction of open channels]), V_m is the membrane potential, and E_{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_{Na} by altering γ 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 γ.

The shifts of the activation and inactivation of I_{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:

$$V_{eff} = V_m + (\psi_i - \psi_o) \tag{3}$$

in which V_{eff} is the overall effective potential across the membrane, V_m is the membrane potential measured in the bulk solutions inside and outside of the sarcolemma, and ψ_i and ψ_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 ψ_o and reduce the difference between ψ_o and ψ_i,

causing a partial depolarization of V_{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 ψ_o causing a partial hyperpolarization of V_{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_{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):

$$\sigma^2 = 2\epsilon\epsilon_o RT \, \Sigma \, c_i[\exp(-z_i\psi_o F/RT) - 1] \quad (4)$$

where σ is the surface charge density, ψ_o is the surface potential, ϵ is the dielectric constant for aqueous solution, ϵ_o is the permittivity of free space, c_i is the concentration of the ith ion in the bulk solution, and z_i is the valence of the ith ion. When an anionic amphiphile is added, σ will be changed after the absorption of the anion to the membrane according to:

$$\sigma' = (1/K)(\sigma'_{max} - \sigma')[A^-]_{x=0} \quad (5)$$

where σ' is the charge density of the amphiphiles absorbed per unit area, σ'_{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:

$$[A^-]_{x=0} = [A^-]_{x=\infty} \exp(\psi_o F/RT) \quad (6)$$

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_{Na} was progressively shifted in the negative direction as the concentration of SDS was increased from 20 to 100 μM, 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_{Na} by SDS is also consistent with the idea that the effects of SDS on I_{Na} are mediated via changes in surface potential (Fig. 8 *A*) 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 ψ_o is reduced by the screening effects of Ca^{2+} since the insertion of SDS is also a function of ψ_o (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 ψ_o. This seems to be the case in this study because: (*1*) the shift of I_{Na} inactivation curve by SDS showed strong concentration dependence despite an increase of ψ_o 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 [Ca^{2+}] despite a decrease in the negativity of ψ_o due to the screening effect of Ca^{2+}, which would be expected to facilitate SDS insertion.

Besides the effects on the amplitude and steady-state gating of I_{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*). τ_h 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_{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_{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 $I_{Ca,L}$ were similarly shifted by SDS and DDTMA by ~10 mV (Ji et al., 1990; Post et al., 1991). This study shows that the shift in $V_{1/2}$ of the I_{Na} activation curve (~10 mV) was less pronounced than that of the steady-state inactivation (~20 mV) or the time constants of the current inactivation (~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_{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 sulphonic 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_{Na} to surface potential alteration. However, this seems inconsistent with the substantial evidence that I_{Na} inactivation is not intrinsically voltage dependent, and that its apparent voltage dependence is related to the voltage dependence of I_{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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REFERENCES

Armstrong, C. M. 1981. Sodium channels and gating currents. *Physiological Reviews.* 61:644–683.

Armstrong, C. M., and F. Bezanilla. 1977. Inactivation of the sodium channel. II. Gating current experiments. *Journal of General Physiology.* 70:567–590.

Armstrong, C. M., and G. Cota. 1990. Modification of sodium channel gating by lanthanum. Some effects that cannot be explained by surface charge theory. *Journal of General Physiology.* 96:1129–1140.

Armstrong, C. M., and D. R. Matterson. 1986. The role of calcium ions in the closing of K channels. *Journal of General Physiology.* 87:817–832.

Balser, J. R., P. B. Bennett, and D. M. Roden. 1990. Time-dependent outward current in guinea pig ventricular myocytes. Gating kinetics of the delayed rectifier. *Journal of General Physiology.* 96:835–863.

Barchi, R. L. 1988. Probing the molecular structure of the voltage-dependent sodium channel. *Annual Review of Neuroscience.* 11:455–495.

Burt, J. M., and G. A. Langer. 1983. Ca^{2+} displacement by polymyxin B from sarcolemma isolated by 'gas-dissection' from cultured neonatal rat myocardial cells. *Biochimica et Biophysica Acta.* 729:44–52.

Cahalan, M. D., and P. A. Pappone. 1981. Chemical modification of sodium channel surface charges in frog skeletal muscle by trinitrobenzene sulphonic acid. *Journal of Physiology.* 321:127–139.

Campbell, D. T., and R. Hahin. 1984. Altered sodium and gating current kinetics in frog skeletal muscle caused by low external pH. *Journal of General Physiology.* 84:771–788.

Catterall, W. A. 1988. Structure and function of voltage-sensitive ion channels. *Science.* 242:50–61.

Cevc, G. 1990. Membrane electrostatics. *Biochimica et Biophysica Acta.* 1031:311–382.

Cota, G., and C. M. Armstrong. 1989. Sodium channel gating in clonal pituitary cells. The inactivation step is not voltage-dependent. *Journal of General Physiology.* 94:213–232.

Cukierman, S., W. C. Zinkand, R. J. French, and B. K. Krueger. 1988. Effects of membrane surface charge and calcium on the gating of rat brain sodium channels in planar bilayers. *Journal of General Physiology.* 92:431–447.

Frankenhauser, B., and A. L. Hodgkin. 1957. The action of calcium on the electrical properties of squid axons. *Journal of Physiology.* 137:218–244.

Giles, W., T. Nakajima, K. Ono, and E. F. Shibata. 1989. Modulation of the delayed rectifier K^+ current by isoprenaline in bull-frog atrial myocytes. *Journal of Physiology.* 415:233–249.

Gilly, W. F., and C. M. Armstrong. 1982. Slowing of sodium channel opening kinetics in squid axon by extracellular zinc. *Journal of General Physiology.* 79:935–964.

Graham, D. C. 1947. The electrical double layer and the theory of electrocapillarity. *Chemical Reviews.* 41:441–501.

Green, W. N., and O. S. Andersen. 1991. Surface charges and ion channel function. *Annual Review of Physiology.* 53:341–359.

Green, W. N., L. B. Weiss, and O. S. Andersen. 1987. Batrachotoxin-modified sodium channels in planar lipid bilayers. Ion permeation and block. *Journal of General Physiology.* 89:841–872.

Hahin, R., and D. T. Campbell. 1983. Simple shifts in the voltage dependence of sodium channel gating caused by divalent cations. *Journal of General Physiology.* 82:785–805.

Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv.* 391:85–100.

Helenius, A., D. R. McCaslin, E. Fries, and C. Tanford. 1979. Properties of detergents. *Methods in Enzymology.* 56:734–749.

Hille, B. 1992. Ionic Channels of Excitable Membranes. 2nd ed. Sinauer Associates, Inc., Sunderland, MA. 607 pp.

Hille, B., A. M. Woodhull, and B. I. Shapiro. 1975. Negative surface charge near the sodium channels of nerve: divalent ions, monovalent ions, and pH. *Philosophical Transactions of the Royal Society of London, Series B.* 270:301–318.

Hodgkin, A. L., and A. F. Huxley. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *Journal of Physiology.* 117:500–544.

Ji, S., J. N. Weiss, and G. A. Langer. 1990. Effect of amphiphiles on calcium currents in rabbit ventricular myocytes. *Biophysical Journal.* 57:526*a*. (Abstr.)

Ji, S., J. N. Weiss, and G. A. Langer. 1992. Charged amphiphiles alter steady-state gating of ionic currents in cardiac ventricular myocytes. *Biophysical Journal.* 61:394*a*. (Abstr.)

Kornfeld, R., and S. Kornfeld. 1985. Assembly of asparagine-linked oligosaccharides. *Annual Review of Biochemistry.* 54:631–664.

Kyte, J., and R. F. Doolittle. 1982. A simple method for displaying the hydropathic character of protein. *Journal of Molecular Biology.* 157:105–132.

Langer, G. A., and T. L. Rich. 1986. Augmentation of sarcolemmal Ca^{2+} by anionic amphiphile: contractile responses of three ventricular tissues. *American Journal of Physiology.* 250 (*Heart and Circulatory Physiology* 19):H247–H254.

Levinson, S. R., W. B. Thornhill, D. S. Duch, E. Recio-Pinto, and B. W. Urban. 1990. The role of nonprotein domains in the function and synthesis of voltage-gated sodium channels. *In* Ion Channels. T. Narahashi, editor. Plenum Publishing Corp., New York. 33–64.

Mato, J. M. 1990. Phospholipid Metabolism in Cellular Signalling. CRC Press, Inc., Boca Raton, FL. 145 pp.

Matsuura, H., T. Ehara, and Y. Imoto. 1987. An analysis of the delayed outward current in single ventricular cells of the guinea pig. *Pflügers Archiv.* 410:596–603.

McLaughlin, S. 1977. Electrostatic potentials at membrane-solution interfaces. *Current Topics in Membranes and Transport.* 9:71–144.

McLaughlin, S., G. Szabo, and G. Eisenman. 1971. Divalent ions and the surface potential of charged phospholipid membrane. *Journal of General Physiology.* 58:667–687.

Mitra, R., and M. Morad. 1985. A uniform enzymatic method for dissociation of myocytes from hearts and stomachs of vertebrates. *American Journal Physiology.* 249 (*Heart and Circulatory Physiology* 18):H1056–H1060.

Nillius, B. 1988. Calcium block of guinea pig heart sodium channels with and without modification by the piperazinylindole DPI 201-106. *Journal of Physiology.* 399:537–558.

Perozo, E., F. Bezanilla, and R. Dipolo. 1989. Modulation of K channels in dialyzed squid axons. ATP-mediated phosphorylation. *Journal of General Physiology.* 93:1195–1218.

Philipson, K. D., G. A. Langer, and T. L. Rich. 1985. Charged amphiphiles regulate heart contractility and sarcolemma-Ca^{2+} interactions. *American Journal of Physiology.* 248(*Heart and Circulatory Physiology* 17):H147–H150.

Post, J. A., S. Ji, K. S. Leonards, and G. A. Langer. 1991. Effects of charged amphiphiles on cardiac cell contractility are mediated via effects on Ca^{2+} current. *American Journal of Physiology.* 260(*Heart and Circulatory Physiology* 29):H759–H769.

Sammar, M., G. Spira, and H. Meiri. 1992. Depolarization exposes the voltage sensor of the sodium channels to the extracellular region. *Journal of Membrane Biology.* 125:1–11.

Sanguinetti, M. C., and N. K. Jurkiewicz. 1990. Lanthanum blocks a specific component of I_K and screens surface charge in cardiac cells. *American Journal of Physiology.* 259(*Heart and Circulatory Physiology* 28):H1881–H1889.

Storch, J., and A. M. Kleinfeld. 1985. The lipid structure of biological membrane. *Trends in Biological Science.* 10:418–421.

Stühmer, W., F. Conti, H. Suzuki, X. Wang, M. Noda, N. Yahagi, H. Kubo, and S. Numa. 1989. Structural parts involved in activation and inactivation of the sodium channel. *Nature.* 339:597–603.

Yamamoto, D., J. Z. Yeh, and T. Narahashi. 1984. Voltage-dependent calcium block of normal and tetramethrin-modified single sodium channels. *Biophysical Journal.* 45:337–344.

Yamamoto, D., J. Z. Yeh, and T. Narahashi. 1985. Interactions of permeant cations with sodium channels of squid axon membranes. *Biophysical Journal.* 48:361–368.

Yee, H. F., J. N. Weiss, and G. A. Langer. 1989. Neuraminidase selectively enhances transient Ca^{2+} current in cardiac myocytes. *American Journal of Physiology.* 256(*Cell Physiology* 25):C1267–C1272.

Zhang, J.-F., and S. A. Siegelbaum. 1991. Effects of external protons on single cardiac sodium channels from guinea pig ventricular myocytes. *Journal of General Physiology.* 98:1065–1083.