Ethanol is known to exert significant effects on the electrical characteristics of the myocardium. A shortening of the action potential with lowering of its plateau during exposure to ethanol has been reported in various cardiac tissue preparations (1–3). However, little information is available regarding the effects of ethanol on transmembrane currents in cardiac muscle. In the present study, we investigated its effects on action potential variables and membrane currents using single electrode current and voltage clamp techniques in enzymatically dispersed single cells from bullfrog atria. The enzymatic dispersion procedure for isolating single atrial cells and the experimental setup for recording membrane potential and membrane current were essentially the same as those described by Hume and Giles (4, 5). The right atrium removed from the bullfrog (Rana catesbeiana) was incubated in Ca\(^{2+}\)-free Ringer’s solution containing 0.15% collagenase and 0.1% trypsin. Single atrial cells were obtained by stirring this incubation medium for 2 hr. They were transferred into a standard Ringer’s solution, which was kept saturated with 95% O\(_2\) and 5% CO\(_2\) (pH 7.4) and contained (mM): NaCl, 90.6; KCl, 2.5; CaCl\(_2\), 2.5; MgCl\(_2\), 5.0; NaHCO\(_3\), 20.0; and glucose, 10.0. Glass microelectrodes filled with 3M KCl and having resistances of 1–5 M\(\Omega\) were introduced into the isolated cells by suction. Membrane potentials were recorded by a conventional DC-amplifier with high input impedance and membrane currents were measured by a patch clamp/whole cell clamp amplifier. The constant application of suction to the microelectrode seemingly minimized leakage of the electrolytes from the tip, since stable resting potentials were recorded for periods of at least 30 min and the reversal potential for slow outward current remained stable and very near the calculated \(E_k\) (see below). Ethanol was dissolved into Ringer’s solution and applied to the cell by perfusion. The temperature of all solutions was kept constant throughout the experiment at 19–22°C.

Isolated single atrial cells remained quiescent in the standard Ringer’s solution. The mean value of the resting membrane potentials was \(-89\pm1.2\) mV (mean±S.D., n=11). Action potentials were induced by brief intracellular stimuli (0.5 msec, 5–10 nA, 0.2 Hz). Exposure to ethanol at concentrations between 100 and 500 mM produced a shortening and lowering of the plateau and a prolongation of the terminal phase of repolarization in a completely reversible manner. The resting potentials slightly decreased by 2–4 mV. These effects were larger and clearly seen at a higher concentration of ethanol (Fig. 1). At 500 mM ethanol, the overshoot of action potential was decreased from 34±1.2 mV to 28±2.1 mV, and the maximum upstroke velocity \(V_{\text{max}}\) was reduced from 24±2.0 V/sec to 16±2.1 V/sec (n=6). They did not change at lower concentrations of less than 200 mM.
The ethanol effects observed in our current clamp studies suggested that major changes occur in currents flowing during the plateau and repolarization phases. Therefore, we studied next the effects on membrane currents in the presence of tetrodotoxin (3 μM). The membrane potential was first held at −85 mV (Vh, zero-current potential for the cell used) and then stepped to between −115 and +55 mV. Figure 2 illustrates a result of the experiment of this kind, showing that there were three different ionic currents (5–7); 1. A transient slow inward current, of which the threshold for activation was approximately −50 mV and the maximum activation was obtained at around −10 mV. This current was markedly reduced by verapamil (10 μM) and by Cd^{2+} (1 mM). Thus it was confirmed to be a slow inward current (i_{si}), carried...
mainly by Ca$^{2+}$ ions. The maximum peak amplitude of $i_{si}$ during the control period was $-243\pm 26$ pA ($n=7$). It was reduced to 69% of the control ($-169\pm 7$ pA, $n=7$) after 200 mM and to 26% ($-63\pm 12$ pA, $n=4$) after 500 mM ethanol. The time to peak of this current was slowed and the inactivation was facilitated by ethanol (Fig. 2A). 2. A time-dependent outward current which followed the $i_{si}$ after its termination within 100–150 msec. When the voltage was stepped positive to +20 mV from $V_h$ for 500 msec, there appeared a gradually increasing activation of the outward current. The second voltage steps back to −70 or to −120 mV resulted in either slow outward (to −70 mV) or slow inward (to −120 mV) tail currents (not shown). These combined pulse experiments revealed that this slow outward current reversed between −95 and −100 mV, suggesting that it is carried mainly by K$^+$ ions. Ethanol reduced this delayed outward K$^+$ current ($i_{k_1}$) dose-dependently. The $i_{k_1}$ measured at the end of the 200 msec voltage step to +55 mV was reduced to 70±8.1% of the control ($n=7$) at 200 mM ethanol and to 57±5.8% ($n=4$) at 500 mM. 3. A time-independent background current which exhibited marked inward-going rectification. Larger inward currents were elicited by hyperpolarizing clamp steps negative to $V_h$. This current was increased by increasing extracellular K$^+$, and it was inhibited by Ba$^{2+}$ (250 $\mu$M), but not by Cd$^{2+}$ (1 mM). Therefore, it is carried through inward-going rectifying K$^+$ channels ($i_{k_1}$). Ethanol had no effect on this current at all concentrations.

The major finding of the present study is that ethanol significantly depressed the slow inward Ca$^{2+}$ current ($i_{si}$) in isolated bullfrog atrial cells. It was due to a reduction of the peak amplitude, prolongation of the time to peak and facilitation of the decay of $i_{si}$. This may account for the marked shortening and lowering of the plateau phase of the action potential recorded with a current clamp (5–7). The terminal phase of repolarization was definitely prolonged by ethanol in our preparation. This result is consistent with that of the voltage clamp experiment which shows a decreased $i_v$ during ethanol application. Ethanol at higher concentrations slightly depolarized the membrane. The $i_{k_1}$ which maintains the resting potential, however, was not affected by ethanol, suggesting that the membrane depolarization may be caused by some mechanisms other than $i_{k_1}$ channels (5–7). In addition, since the overshoot as well as $V_{max}$ of the action potential were reduced by relatively high concentrations of ethanol, it is assumed that the alcohol decreases the fast inward Na$^+$ current ($i_{Na}$). Recently, Ikeda et al. (8) have reported that ethanol inhibits $i_{si}$, $i_{Na}$ and $i_v$ in decreasing order determined in bullfrog atrial cells with a double sucrose-gap voltage clamp. The analysis of membrane currents, though it is not yet satisfactory, can explain the basic mechanism of the acute electrophysiological effect of ethanol on the myocardium, including disturbances of intracardiac conduction (1–3).

References