Voltage-Independent Effects of Extracellular K+ on the Na+ Current and Phase 0 of the Action Potential in Isolated Cardiac Myocytes

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Abstract A rise in [K+]o, by depolarizing the resting membrane potential and partially inactivating the inward Na+ current (I\textsubscript{Na}), is believed to play a critical role in slowing conduction during myocardial ischemia. In multicellular ventricular preparations, elevation of [K+]o has been suggested to decrease \( V_{\text{max}} \) to a greater extent than expected from membrane depolarization alone. The mechanism of this voltage-independent effect of [K+]o is currently unknown, and its significance in single cardiac cells has not been determined. We have examined the voltage-independent effects of elevated [K+]o on I\textsubscript{Na} and the action potential upstroke in isolated rabbit atrial and ventricular myocytes under voltage- and current-clamp conditions. Superfusate [K+]o varied from 5 mmol/L to 14 or 24 mmol/L, whereas [Na+] was maintained at 150 mmol/L. In cultured atrial cells and excised outside-out patches from freshly isolated atrial and ventricular cells, the amplitude and kinetics of I\textsubscript{Na} were unchanged by elevation of [K+]o. In atrial cells, action potentials elicited from a holding potential of \(-70 \text{ mV}\) had a similar \( V_{\text{max}} \) (114.9±5.7 versus 112.2±4.8 V/s, mean±SEM, n=6) and action potential amplitude (115.0±2.4 versus 113.4±3.9 mV) in 5 and 24 mmol/L [K+]o. In contrast, in ventricular cells at a holding potential of \(-70 \text{ mV}\), increasing [K+]o, from 5 to 14 mmol/L decreased \( V_{\text{max}} \) from 161.8±18.0 to 55.3±5.0 V/s (n=7, \( P<.001 \)) and action potential amplitude from 128.1±1.3 to 86.6±5.4 mV (\( P<.001 \)). This voltage-independent decrease in \( V_{\text{max}} \) and action potential amplitude induced by elevated [K+]o was abolished in the presence of 1 mmol/L Ba\textsuperscript{2+}, suggesting that it is attributable to an increased background K\textsuperscript{+} conductance. We conclude that elevation of [K+]o, to levels expected during ischemia causes a marked voltage-independent depression of \( V_{\text{max}} \) in ventricular cells, which may, in turn, contribute to the slowing of myocardial conduction characteristic of early ischemia. (Circ Res. 1994;75:491-502.)

Key Words • Na+ • Na+ current • action potential • \( V_{\text{max}} \)

The electrophysiological changes that accompany the onset of acute myocardial ischemia create a milieu conducive to the development of ventricular arrhythmias.\(^1,2\) The earliest change is depolarization of the resting membrane, which is soon followed by a decrease in action potential amplitude and maximal upstroke velocity (\( V_{\text{max}} \)).\(^3,4,5\) These changes in action potential configuration, in turn, are largely responsible for the slowing of impulse conduction, unidirectional block, and reentrant excitation, which are thought to underlie the majority of early (type IA) arrhythmias.\(^6-10\) Of the many metabolic, ionic, and neurohumoral elements present in ischemia that contribute to the changes in action potential configuration, elevation of [K+]o appears to play a central role.\(^1,11-13\) [K+]o, begins to rise within 10 to 20 seconds after experimental coronary occlusion and may reach 15 to 20 mmol/L in the central ischemic zone within 20 to 30 minutes.\(^11,14,15\) The rise in [K+]o, causes depolarization of the resting membrane potential (RMP). Membrane depolarization, in turn, by increasing the proportion of Na+ channels in the inactive state, decreases the fraction of Na+ channels available for activation.\(^15-17\) This voltage-dependent decrease in the transient inward Na+ current (I\textsubscript{Na}) decreases \( V_{\text{max}} \) of the action potential and contributes to the early slowing of myocardial conduction characteristic of ischemia.\(^10,11,18,19\)

A study using cat and guinea pig papillary muscle has suggested that in addition to the voltage-dependent effects of [K+]o on \( V_{\text{max}} \) elevation of [K+]o, to 11.5 to 24.0 mmol/L, depresses \( V_{\text{max}} \) to a greater extent than can be attributed to depolarization of RMP alone.\(^20\) The mechanism of this voltage-independent depression of \( V_{\text{max}} \) by [K+]o is unknown. Kishida et al\(^20\) postulated that it may be due to a direct effect of extracellular K\textsuperscript{+} on the amplitude and kinetics of I\textsubscript{Na} as has been well described in neuronal preparations. In the voltage-clamped squid giant axon, for example, elevation of [K+]o has been reported to decrease the amplitude of I\textsubscript{Na}, cause a leftward shift in the steady-state inactivation relation along the voltage axis, and increase the time constants for the development of and recovery from inactivation.\(^21,22\) In that preparation\(^23\) and in the frog node of Ranvier,\(^24\) increased [K+]o also enhances the rate of development and extent of slow inactivation. In agreement with these findings [K+]o, of 10.8 mmol/L was reported to “depress the excitatory Na+ system” in an early study using a ramp voltage-clamp protocol in shortened cardiac Purkinje fibers.\(^25\)

An alternative explanation for the voltage-independent effect of [K+]o on \( V_{\text{max}} \) is that it may result from a K\textsuperscript{+}-induced increase in the background K\textsuperscript{+} conductance (g\textsubscript{K}) independent of any direct effect on I\textsubscript{Na}, since
elevation of $g_K$ magnifies the nonlinear relation between $I_{Na}$ and $V_{max}$.\textsuperscript{26}

Despite the central role of extracellular $K^+$ in the pathophysiology of myocardial ischemia, there have been no published reports directly examining the effect of $[K']_o$ on $I_{Na}$ in voltage-clamped isolated cardiac myocytes. Moreover, the voltage-independent effects of $[K']_o$ on $V_{max}$ reported in multicellular preparations have not been examined and confirmed at the single-cell level. Therefore, we have examined the voltage-independent effects of $[K']_o$ on $I_{Na}$ and phase 0 of the action potential in isolated atrial and ventricular myocytes. Elevation of $[K']_o$ to levels encountered in the extracellular space during myocardial ischemia has no direct effect on the amplitude or kinetics of $I_{Na}$, $[K']_o$ exerts no voltage-independent effect on $V_{max}$ in atrial myocytes but profoundly decreases $V_{max}$ and action potential amplitude in ventricular myocytes. The effect of $[K']_o$ in ventricular myocytes is attributable to an increase in $g_K$. Preliminary reports of these data have appeared previously.\textsuperscript{27,28}

Materials and Methods

Cell Isolation and Culture

Experiments were performed by using atrial and ventricular myocytes isolated from male New Zealand White rabbits (1.7 to 3.5 kg) by an enzyme-dissociation procedure.\textsuperscript{29} Animals were anesthetized with 5 to 10 mg/kg IM ketamine and 40 to 70 mg/kg IP pentobarbitol. Heparin (600 U) was administered via a marginal ear vein. A tracheostomy was performed, and rabbits were ventilated while the heart was rapidly excised and perfused by the Langendorff technique.

The heart was initially perfused with Ca\textsuperscript{2+}-free Eagle's minimum essential medium (MEM-0518, Sigma Chemical Co) for 5 minutes to clear the coronary arteries and ventricles of residual blood. The preparation was then perfused with the enzyme solution for 30 to 45 minutes (see below for composition). The atria and ventricles were separated and cut into small segments. Isolated myocytes were separated from the tissue segments by filtration through a 220-μm nylon mesh. Myocytes were suspended and stored in a culture medium composed of Ham's F-12 and Dulbecco's modified Eagle's medium (Hyclone Laboratories Inc) in a 1:1 ratio, 10% calf bovine serum, 180 μg/mL streptomycin, and 75 μg/mL mefoxin. The atrial cells were plated onto 18×18-mm coverslips coated with Laminin (Gibco Laboratories); ventricular cells were maintained in suspension in the culture medium. Myocytes were stored in an incubator at 37°C in a CO\textsubscript{2}-enriched humidified atmosphere. Ventricular cells were used within 10 hours of isolation. Atrial myocytes were used after they had assumed a spherical morphology (usually after 2 or 3 days). This shape has been shown to be advantageous for controlling the membrane potential during voltage clamping.\textsuperscript{30} In some experiments, $I_{Na}$ was recorded in freshly isolated atrial cells.

Solutions and Reagents

The solution used for enzymatic dissociation of the Langendorff-perfused heart was minimum essential medium containing 180 U/mL type II collagenase (Worthington Diagnostics), 10 mg/100 mL hyaluronidase (type I-S, Sigma), 92 U/100 mL protease (type XIV, Sigma), 25 to 40 μmol/L CaCl\textsubscript{2}, and 10 mmol/L HEPES. This solution was gassed with 100% O\textsubscript{2}, and the pH was adjusted to 7.4 with NaOH. The standard experimental superfusate was a modified Tyrode's solution containing (mmol/L) NaCl 145, KCl 5.0, CaCl\textsubscript{2} 1.5, MgCl\textsubscript{2} 1.0, HEPES 10, and glucose 5. The pH was adjusted to 7.4 at room temperature (20°C to 22°C) with NaOH. The osmolality of samples of this solution was 291±3 mosm (mean±SEM, n=13). To examine the effects of elevated extracellular K\textsuperscript{+}, KCl was added to the Tyrode's solution without osmotic compensation. Samples of Tyrode's solution containing 14 and 24 mmol/L KCl had osmolalities of 303±4 mosm (n=7) and 317±3 mosm (n=9), respectively (Wescor 5500 Vapour Pressure Osmometer). CdCl\textsubscript{2} (50 μmol/L) was added to all superfusates during recording of $I_{Na}$ to inhibit inward current through Ca\textsuperscript{2+} channels.

To examine the kinetics of $I_{Na}$ in cultured atrial myocytes, we used the perforated-patch technique.\textsuperscript{31} This technique obviates the spontaneous time-dependent shifts in activation and inactivation kinetics encountered with conventional whole-cell voltage clamping and enables recording of $I_{Na}$ with stable kinetics for up to 2 hours.\textsuperscript{32} The internal solution for perforated-patch experiments had the following composition (mmol/L): potassium gluconate 120, CsCl 30, HEPES 5, and EGTA 1. The pH was adjusted to 7.3 with CsOH. All experiments were performed at room temperature (20°C to 22°C).

The internal solution used for whole-cell patch-clamp experiments contained (mmol/L) KCl 30, potassium aspartate 120, KH\textsubscript{2}PO\textsubscript{4} 1.0, Na\textsubscript{2}ATP 5, MgCl\textsubscript{2} 1.0, HEPES 5, and EGTA 5. The pH of this solution was titrated to 7.3 at 20°C with KOH. The osmolality of this solution was 275 mosm. $I_{Na}$ was recorded in freshly isolated atrial and ventricular myocytes by using excised outside-out patches. The pipette solution used in these experiments was identical to that used in whole-cell experiments, with the exception that KCl was replaced by 30 mmol/L CsCl to inhibit K\textsuperscript{+} conductance, and potassium aspartate was replaced by 120 mmol/L KF.

Cells were superfused at 2 mL/min. During exchange of superfusates, [K\textsuperscript{+}] in the tissue bath was continuously monitored by using an ion-selective electrode (Kwik-Tip, World Precision Instruments Inc). [K\textsuperscript{+}] achieved a new steady state within 2 minutes after a switch of superfusates, and recordings were made 10 minutes after the new steady-state [K\textsuperscript{+}] was reached. A 3 mmol/L stock solution of tetrodotoxin was prepared by dissolving 1 mg in 1 mL of the standard Tyrode's solution.

Recording Techniques

Patch micropipettes were fabricated from borosilicate glass tubing (outer diameter, 1.5 mm; N-51A, Drummond Scientific Co) by using a Flaming-Brown P80/PC horizontal puller (Sutter Instrument Co). Micropipettes were coated with Sylgard 184 elastomer (Dow Corning). They were fire-polished immediately before use. In experiments using the perforated-patch technique, we used electrodes with steep tapers and resistances of 700 to 900 kΩ when filled with the internal solution. This helped to minimize series resistance (typically, 3 to 4 MΩ uncompensated).

For experiments using the conventional whole-cell and outside-out patch-clamp configurations, we used micropipettes of 2.0- to 4.0-MΩ resistance. Micropipettes were coupled to the headstage of the patch-clamp amplifiers by a Ag/AgCl wire. The tissue bath was gassed via a Ag/AgCl wire embedded in an agar gel, which had been prepared with the internal solution. This minimized junction potentials to 2 to 6 mV. This offset potential was nulled before the formation of gigahm seals.

Action potentials were measured with a Dagan 3900A patch-clamp amplifier (Dagan Corp) in the current-clamp mode. Signals were filtered at 10 kHz and sampled at 40 kHz to enable accurate determination of $V_{max}$ of the action potential.\textsuperscript{33} Whole-cell membrane currents were measured by using a List L/M EPC-7 patch-clamp amplifier (List Electronics Co). $I_{Na}$ in excised patches from atrial and ventricular cells were measured by using the Dagan 3900 amplifier to enable leakage subtraction. Current signals were filtered at 10 kHz and sampled at 20 kHz.
Data Storage and Analysis

Membrane potential and current signals were monitored on a Tektronics 5113 dual-beam storage oscilloscope (Tektronics Inc). Voltage- and current-clamp stimulation protocols were generated by a Compaq-Prolinea 3/25s personal computer (Compaq Computer Corp) with a TL-1 interface (Axon Instruments) and customized software. Data were digitized with an analog-to-digital interface (DT 2821, Data Translation) and a Compaq 386-20 computer. Digitized data were analyzed off-line with a Sun 4/280 microcomputer.

Experimental Protocols

An 18×18-mm coverslip containing the cultured atrial myocytes was placed in a tissue bath mounted on the stage of a Nikon-Diaphot-TMD inverted microscope (Nippon Kogaku). For experiments using freshly isolated atrial and ventricular cells, the base of the tissue bath was coated with laminin. Several drops of the cell suspension were placed in the bath. After allowing the cells to settle and adhere to the base for 15 to 20 minutes, they were superfused at 2 mL/min.

After achieving the whole-cell configuration, action potentials were elicited in the current-clamp mode by applying brief (1-millisecond) depolarizing current pulses. Stimulus strength was adjusted to produce a latency of 2 to 2.5 milliseconds. This generally required stimulus pulses at 1.2 to 1.5 times diastolic threshold. Cells were stimulated at 0.33 Hz. Details of the technique used to record Na⁺ currents from atrial myocytes by the amphotericin perforated-patch method have been described previously.32 Excised outside-out patches were obtained according to the method of Hamill et al.34 In voltage-clamp experiments, capacitative transients were canceled, and series resistance compensation was applied. Typically, 80% to 90% compensation could be achieved. The net voltage error was calculated from the peak inward Na⁺ current and the residual series resistance.35 Only cells in which the voltage error was <3.5 mV were analyzed. If any threshold phenomenon was observed in the negative limb of the current-voltage (I-V) curve, the experiment was abandoned.

Data Analysis and Statistics

Activation and inactivation curves for Na⁺ currents were fitted to standard Boltzmann functions. A similar procedure was used to fit exponentials to the time courses of the recovery from inactivation and the development of slow inactivation. Data are expressed as mean±SEM. Statistical comparisons were made by using Student’s t test for paired data and ANOVA. Differences between means were regarded as statistically significant at P<.05.

Results

I_K, in Atrial Myocytes During Exposure to Elevated [K⁺].

The kinetics of I_K, in cultured atrial myocytes were examined during exposure to solution containing 5 and 24 mmol/L [K⁺]. We chose 24 mmol/L [K⁺] because this [K⁺], represents the upper range of values reported in the extracellular space during the reversible phase of ischemia.31 The I-V relation was determined using 30-millisecond depolarizing pulses applied at 2-second intervals from a holding potential of −100 mV. The pulses were incremented in 5-mV steps from −80 to +25 mV. Fig 1A shows the current tracings from a representative experiment and the corresponding I-V curves. In the example shown, elevation of [K⁺], produced a slight positive shift in the I-V relation along the voltage axis but had no effect on the peak value of I_K. In seven cells, elevation of [K⁺], from 5 to 24 mmol/L caused no significant change in peak I_K amplitude (6.39±1.00 versus 6.30±1.03 nA), activation midpoint (−37.5±0.8 versus −38.4±0.7 mV), or slope factor.
(8.7±1.3 versus 8.1±1.7 mV). To examine whether the lack of effect of [K+]o on INa amplitude could be related to changes imposed by maintaining the atrial cells in culture, we examined the I-V relation in excised outside-out patches from freshly isolated cells exposed to 5 and 24 mmol/L [K+]o. These experiments confirmed the [K+]o insensitivity of INa found in the cultured atrial cells. In six experiments, peak INa was 125±32 pA in 5 mmol/L [K+]o, and 121±33 pA in 24 mmol/L [K+]o, (P=NS). The potential at which peak INa occurred was -30.0±3.3 and -32.2±2.8 mV in 5 and 24 mmol/L [K+]o, respectively (P=NS).

Steady-state inactivation curves were derived using a 500-millisecond conditioning pulse followed by a test potential to -20 mV. The conditioning potential was incremented in 5-mV steps from -120 to -30 mV. Fig 1B shows current tracings and the inactivation curve derived from the same cell as in Fig 1A. The data have been fitted to a Boltzmann function. In seven experiments, the potential for half inactivation of INa was -70.9±1.7 mV in 5 mmol/L [K+]o, and -72.1±1.6 mV in 24 mmol/L [K+]o, (P=NS). Slope factors were also unchanged (6.1±0.2 versus 6.1±0.2 mV).

Both fast and slow Na+ channel inactivation are known to be sensitive to [K+]o in the squid giant axon and frog node of Ranvier. 22,24 We examined the effect of [K+]o on the kinetics of these processes in atrial myocytes. Recovery of INa from fast inactivation was studied by using a two-pulse protocol. From a holding potential of -100 mV, a conditioning pulse to -20 mV was applied for 150 milliseconds. The cell was then clamped at the holding potential for a variable recovery interval followed by a test pulse to -20 mV. Fig 2A shows INa plotted as a function of the recovery interval in a typical experiment. INa amplitude is expressed as a fraction of the peak INa elicited by a control pulse to -20 mV. Recovery curves were best fitted by a monoexponential function as has been previously reported in the rabbit atrium. 35 The time constants of recovery of INa derived from the fitted curves in seven experiments were 10.2±1.0 and 10.8±1.1 milliseconds in 5 and 24 mmol/L [K+]o, respectively (P=NS).

Slow inactivation was studied by increasing the duration of the conditioning pulse. From a holding potential of -100 mV, prolonged depolarizing conditioning potentials (200 milliseconds to 25 seconds) to -20 mV were applied to induce slow inactivation of the Na+ channel. This was followed by a return to -100 mV for 200 milliseconds to allow recovery from fast inactivation. The recovery phase was followed by a final test pulse to -20 mV. Fig 2B shows data from a representative experiment. The kinetics of slow inactivation remain stable during exposure to elevated [K+]o. In six experiments, the time constants for the development of slow inactivation at -20 mV were 13.0±2.2 seconds in 5 mmol/L [K+]o, and 14.3±2.2 seconds in 24 mmol/L [K+]o, (P=NS). After a 25-second conditioning pulse, the fraction of INa remaining free from slow inactivation was insensitive to an increase in [K+]o, (0.79±0.01 versus 0.78±0.01). The insensitivity of the kinetics of INa to elevated [K+]o, in atrial cells was confirmed by experiments in an additional four cells exposed to 14 mmol/L [K+]o.

INa in Ventricular Myocytes During Exposure to Elevated [K+]o.

The spherical shape (diameter, 15 to 20 μm) and small membrane capacitance (Cm, 15 to 25 pF) of cultured atrial myocytes allowed INa to be voltage-clamped in 150 mmol/L Na+ superfusate. In contrast, in the much larger ventricular myocyte (Cm, ∼100 to 140 pF), it is not possible when using a single electrode voltage clamp to control INa under similar conditions. To examine INa in ventricular myocytes during exposure to physiological [Na+]o, (150 mmol/L), we used the excised outside-out patch configuration. To minimize the poten-
tially confounding effects of the rapid negative shift in voltage dependence of \( I_{Na} \) in this configuration, data collection was begun 5 minutes after patch excision. An abbreviated protocol was used to examine the I-V relation in view of the relatively short-term stability of these patches. From a holding potential of \(-130 \) mV, test pulses were applied in increments of \( 10 \) mV from \(-90 \) to \(-20 \) mV. Since there were relatively few \( Na^+ \) channels per patch, 10 voltage-clamp pulses were applied at each potential and then averaged to improve the signal-to-noise ratio. Fig 3 shows \( I_{Na} \) obtained from an outside-out membrane patch during exposure to 5 and 14 mmol/L \([K^+]_o\) and the resulting I-V relation. Each current tracing represents the average of 10 voltage-clamp pulses at a given potential. Peak \( I_{Na} \) was unaffected by elevation of \([K^+]_o\). In six experiments, peak \( I_{Na} \) was 291±38 pA in 5 mmol/L \([K^+]_o\) and 295±37 pA in 14 mmol/L \([K^+]_o\) (\( P=NS \)).

**\( V_{\text{max}} \) in Atrial Myocytes During Exposure to Elevated \([K^+]_o\)**

Having determined that extracellular \( K^+ \) does not directly affect the cardiac \( Na^+ \) current, we next examined whether \([K^+]_o\) has any voltage-independent effect on phase 0 of the action potential in isolated myocytes under current-clamp conditions. The voltage-independent effects of elevated \([K^+]_o\) on \( V_{\text{max}} \) were first examined in atrial myocytes. In control Tyrode’s solution, the mean RMP was \(-66.4\pm1.3 \) mV (\( n=14 \)). This is in close agreement with previously reported values in rabbit atrial myocytes recorded under similar conditions.\(^{36}\) During exposure to 24 mmol/L \([K^+]_o\), RMP depolarized to \(-33.2\pm1.6 \) mV. To examine the voltage-independent effects of elevated \([K^+]_o\) on \( V_{\text{max}} \), action potentials were elicited with 1-millisecond depolarizing current pulses from a constant membrane potential of \(-70 \) mV. This required application of a hyperpolarizing holding current of \( 10 \) pA in 5 mmol/L \([K^+]_o\), and \( 54 \) pA in 24 mmol/L \([K^+]_o\). The threshold current required to initiate a regenerative action potential was \( 480\pm70 \) pA in 5 mmol/L \([K^+]_o\), and \( 565\pm68 \) pA in 24 mmol/L \([K^+]_o\). Fig 4 shows the action potential upstroke and its first time derivative (\( dV/dt \)) from an atrial cell under control conditions and during exposure to 24 mmol/L \([K^+]_o\). There is no effect of elevated \([K^+]_o\) on \( V_{\text{max}} \) or action potential amplitude recorded at a holding potential of \(-70 \) mV. In six experiments, \( V_{\text{max}} \) was \( 114.9\pm5.7 \) and \( 112.2\pm4.8 \) V/s in 5 and 24 mmol/L \([K^+]_o\), respectively (\( P=NS \)). Action potential amplitude was also unchanged by elevation of \([K^+]_o\) (\( 115.0\pm2.4 \) versus \( 113.4\pm3.9 \) V, \( P=NS \)).

**Fig 3.** A, Recordings show \( Na^+ \) current \( (i_{Na}) \) in an excised outside-out ventricular patch exposed to 5 and 14 mmol/L \([K^+]_o\). \([Na^+]_o \) was 150 mmol/L. From a holding potential of \(-130 \) mV, voltage-clamp steps were applied in 10-mV increments from \(-90 \) to \(-20 \) mV. B, Graph shows voltage-clamp protocol and the derived current-voltage relation.

**Fig 4.** Voltage-independent effect of \([K^+]_o\) on the atrial action potential upstroke. Upper panels show action potential upstrokes elicited from a holding potential of \(-70 \) mV during exposure to 5 mmol/L (left) and 24 mmol/L (right) \([K^+]_o\). Action potentials were elicited with 1-millisecond depolarizing current pulses. Lower panels show first time derivatives (\( dV/dt \)) corresponding to the action potential upstrokes. \( V_{\text{max}} \) was \( 105 \) V/s in 5 mmol/L \([K^+]_o\) and 106 V/s in 24 mmol/L \([K^+]_o\) solution.
Therefore, 

\[ [K^+]_o \]

were elicted from a holding potential of \(-70 \) mV. The current required to clamp the membrane at this potential was \(32 \pm 6.7 \) pA in control and \(1225 \pm 196 \) pA in \(14 \) mmol/L \([K']_o \) solution. The threshold currents required to initiate a regenerative action potential with a 1-millisecond stimulus duration were \(3.6 \pm 0.4 \) and \(5.0 \pm 0.8 \) nA in normal and high \([K']_o \), respectively. The takeoff potential was similar at the two \([K']_o \) levels \((-38.0 \pm 1.9 \) versus \(-37.7 \pm 1.9 \) mV). Fig 5 shows tracings from a representative experiment. In contrast to the findings in atrial cells, elevation of \([K']_o \), substantially reduced \(V_{max} \) and action potential amplitude in ventricular cells. In seven experiments, \(V_{max} \) decreased from \(161 \pm 18.0 \) V/s at 5 mmol/L \([K']_o \) to \(55.3 \pm 5.0 \) V/s during exposure to \(14 \) mmol/L \([K']_o \), \(P<.001 \). This represents a \(66 \pm 7 \% \) reduction in \(V_{max} \). Action potential amplitude decreased from \(128.0 \pm 1.3 \) to \(86.6 \pm 5.4 \) mV, respectively \(P<.001 \). This represents a \(32 \pm 4 \% \) decrease in action potential amplitude. Studies in multicellular cardiac preparations have found no voltage-independent effect of \([K']_o \) on \(V_{max} \) at concentrations \(<10 \) mmol/L.

**Effect of Holding Current on \(V_{max} \) in Ventricular Myocytes**

In the standard current-clamp protocol used to elicit action potentials, the outward current \(I_h \) required to hold the RMP at \(-70 \) mV was maintained during and after the action potential upstroke. To initiate action potentials, a depolarizing stimulus pulse was superimposed on \(I_h \). This protocol is illustrated in Fig 6A. \(I_h \) was significantly larger in \(14 \) mmol/L \([K']_o \) than in control.
solution because of the accompanying membrane depolarization and increase in \( g_k \). This larger \( I_s \) resulted in faster repolarization during exposure to 14 mmol/L \([K^+]_o\) than during exposure to 5 mmol/L \([K^+]_o\), a finding consistent with the study of Kishida et al\(^{20}\) in isolated papillary muscle. We examined whether the difference in \( I_s \) also contributed to the depression of \( V_{\text{max}} \) in 14 mmol/L \( K^+ \) solution. In four additional cells, experiments were performed by using both the standard protocol and a modified protocol (see Fig 6B) in which \( I_s \) was applied for 500 milliseconds and then switched off simultaneously with the application of a 1-millisecond depolarizing pulse. Thus, no \( I_s \) was applied during the upstroke. The stimulus strength was adjusted to produce action potentials with latencies similar to those elicited by the standard protocol. As illustrated in Fig 6, \( V_{\text{max}} \) derived from the standard and modified protocols was similar in solutions containing 5 or 14 mmol/L \([K^+]_o\). In four experiments, \( V_{\text{max}} \) in 5 mmol/L \([K^+]_o\) solution was 181.8±31.0 V/s when the standard protocol was used and 182.9±26.6 when the modified protocol was used. In 14 mmol/L \([K^+]_o\), \( V_{\text{max}} \) was 101.0±5.9 and 99.7±6.7 V/s, respectively. The mean depression of \( V_{\text{max}} \) by 14 mmol/L \([K^+]_o\) was 44.0±11.0% when the standard protocol was used and 45.5±9.0% when the modified protocol was used. Voltage-independent depression of \( V_{\text{max}} \) and action potential amplitude by \([K^+]_o\) therefore cannot be attributed to an increase in \( I_s \) applied during the action potential upstroke.

**Response of Steady-State I-V Relation and Membrane Resistance to \([K^+]_o\) in Atrial and Ventricular Myocytes**

The response of \( V_{\text{max}} \) to elevated \([K^+]_o\) in atrial and ventricular cells is markedly different. This cannot be ascribed to a differential sensitivity of the \( Na^+ \) channel to \([K^+]_o\) (Figs 1 through 3). However, it may relate to differences in the magnitude of \( g_k \) and hence the \( K^+ \) sensitivity of membrane resistance between the two cell types. Such differences are suggested by the relative size of the threshold current required to elicit action potentials in atrial (200 to 300 pA) compared with ventricular (3 to 4 nA) myocytes and in the shape and amplitude of the passive membrane response to the stimulating current pulse (compare Figs 4 and 5). To examine this possibility, membrane resistance \( (R_m) \) was determined at \(-70 \text{ mV} \) in atrial and ventricular cells during exposure to 5 mmol/L \([K^+]_o\), and either 14 or 24 mmol/L \([K^+]_o\). \( R_m \) was estimated from the electrotonic membrane voltage response generated by a small hyperpolarizing current step. Stimulus strength was adjusted to produce a voltage response of \(-5 \text{ mV} \). In atrial cells, \( R_m \) was 958±158 \( \Omega \) in 5 mmol/L \([K^+]_o\) and 768±239 \( \Omega \) in 24 mmol/L \([K^+]_o\) \( (P=\text{NS}, n=6) \). In ventricular cells, \( R_m \) was much smaller, measuring 29.7±3.8 \( \Omega \) in 5 mmol/L \([K^+]_o\). \( R_m \) decreased to 17.1±2.8 \( \Omega \) during exposure to 14 mmol/L \([K^+]_o\). This decrease in \( R_m \) \((42.4±3.0\%)\) is in agreement with the 40.2% decrease predicted from previous studies.\(^{38,39}\) The membrane steady-state I-V relation and its response to elevated \([K^+]_o\) was examined in atrial and ventricular cells. Experiments were performed in the presence of 20 to 30 \( \mu \text{mol/L} \) tetrodotoxin and 100 \( \mu \text{mol/L} \) CdCl\(_2\) to inhibit \( Na^+ \) and Ca\(^{2+} \) conductances, respectively. Voltage-clamp pulses (500 milliseconds) were applied from a holding potential of \(-50 \text{ mV} \). The pulses were incremented in 10-mV steps from \(-130 \text{ to } +50 \text{ mV} \). Fig 7 compares the I-V relation in an atrial and ventricular cell determined from the steady-state current level at the end of the 500-millisecond pulse. In the atrial cell exposed to 5 mmol/L \([K^+]_o\), the relation is relatively flat and has no clear region of negative slope conductance. This is consistent with the high value of \( R_m \) measured in earlier experiments. Indeed, \( R_m \) determined from the slope of the linear portion of the I-V relation, correlated well with the value obtained when using hyperpolarizing current pulses (see above). Exposure to 24 mmol/L \([K^+]_o\) produced a small increase in inward current at potentials negative to the \( K^+ \) equilibrium potential but no significant change in outward current. In contrast, the membrane I-V relation in the ventricular cell exposed to 5 mmol/L \([K^+]_o\), is steep and inwardly rectifying with a prominent region of negative slope conductance between \(-40 \text{ and } 0 \text{ mV} \). In 14 mmol/L \([K^+]_o\), the relation is shifted in the positive direction on the voltage axis, and the peak outward current is increased. As previously reported, there is significant crossover of the I-V relation in normal and high \([K^+]_o\). \(^{36}\) The marked
differences in the amplitude of the background $g_K$ and hence the passive membrane properties of atrial and ventricular cells may confer to the latter cells a greater sensitivity to $[K^+]_o$.

**Ba$^{2+}$ and the Voltage-Independent Effects of $[K^+]_o$ on $V_{max}$**

To directly examine the role of increased $g_K$ in the voltage-independent depression of $V_{max}$ in ventricular cells, experiments were performed during exposure to Ba$^{2+}$, an inhibitor of $g_K$. In the presence of 1 mmol/L [Ba$^{2+}$], the ventricular I-V relation was nearly linearized (see Fig 8). Fig 9 shows action potentials elicited from a holding potential of $-70$ mV and the corresponding $dV/dt$ derived from a ventricular cell exposed to solution containing 5 mmol/L [K$^+$] and solutions containing 1 mmol/L [Ba$^{2+}$] and either 5 or 14 mmol/L [K$^+$]. There was no significant difference in $V_{max}$ or action potential amplitude during exposure to any of the three solutions. In a total of four experiments performed in the presence of Ba$^{2+}$, $V_{max}$ was $157.9 \pm 19.9$ V/s in 5 mmol/L [K$^+$], and $158.3 \pm 16.8$ V/s in 14 mmol/L [K$^+$], solution. Action potential amplitude was $130.1 \pm 2.2$ and $130.3 \pm 1.8$ mV, respectively. Inhibition of $g_K$ with Ba$^{2+}$ therefore abolishes the voltage-independent depression of $V_{max}$ induced by [K$^+$].

**Computer Simulations Using the Luo-Rudy Ventricular Model**

To provide a theoretical basis for interpretation of the experimental results, we have simulated similar protocols by using the Luo-Rudy mathematical model\textsuperscript{40} of the ventricular action potential. This model incorporates the option of changing [K$^+$], and considers its effects on the conductances of the membrane K$^+$ currents, $I_K$ and $I_{Na}$. Fig 10 shows the computed rising phase of the action potential (top), its first derivative ($dV/dt$, middle), and $I_{Na}$ (bottom) for two values of [K$^+$]: 5 mmol/L (left) and 14 mmol/L (right). Simulation protocols are described in the figure legend. The following effects are observed as a result of the increase in [K$^+$], to 14 mmol/L: (1) $V_{max}$ decreases from 156.3 to 134.4

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**Fig 8.** Graph showing the effect of Ba$^{2+}$ on the steady-state current-voltage (I-V) relation in a ventricular myocyte. By use of the same protocol as in Fig 7, the background I-V relation was determined during exposure of a ventricular myocyte to solutions containing 5 mmol/L [K$^+$], 14 mmol/L [K$^+$], and 14 mmol/L [K$^+$]+1 mmol/L [Ba$^{2+}$]. Note that in the presence of Ba$^{2+}$, the I-V relation is nearly linearized.

**Fig 9.** Effect of Ba$^{2+}$ on the voltage-independent depression of action potential upstroke by [K$^+$], in a ventricular myocyte. Upper panels show action potential upstrokes elicited from a ventricular myocyte at a holding potential of $-70$ mV. The myocyte was exposed to 5 mmol/L K$^+$ Tyrode's solution (left), Tyrode's solution containing 5 mmol/L [K$^+$] and 1 mmol/L [Ba$^{2+}$] (middle), and solution containing 14 mmol/L [K$^+$] and 1 mmol/L [Ba$^{2+}$] (right). The corresponding $dV/dt$ values are indicated in the lower panels. Ba$^{2+}$ abolished the voltage-independent effects of [K$^+$], on $V_{max}$ and action potential amplitude.
ingly, membrane voltage was stepped to a more positive peak potential during the stimulus pulse than in 5 mmol/L \([K^{+}]_o\), resulting in greater inactivation of \(I_{Na}\) and a lower value of parameter \(h\) at the onset of the action potential upstroke and at \(V_{max}\). Thus, the Luo-Rudy model\(^{40}\) suggests that at least part of the voltage-independent effect of \([K^{+}]_o\) on \(V_{max}\) is due to a change in the passive properties of the membrane (a decrease in \(R_m\)), which, in turn, influences the history of the membrane before the action potential upstroke and the availability of \(Na^{+}\) channels for activation during the upstroke.

**Discussion**

**Major Findings**

The present study has examined the voltage-independent (direct) effects of elevated \([K^{+}]_o\), on \(I_{Na}\) and phase 0 of the action potential in isolated atrial and ventricular myocytes. We find that at values of \([K^{+}]_o\), that encompass the range encountered during myocardial ischemia, the kinetics and amplitude of \(I_{Na}\) remain constant. Although \(I_{Na}\) studied under voltage clamp in both atrial and ventricular myocytes is insensitive to moderate elevation of \([K^{+}]_o\), the response of \(V_{max}\) is vastly different in the two cell types. \([K^{+}]_o\) has no significant voltage-independent effect on \(V_{max}\) in the cultured atrial cells but markedly depresses \(V_{max}\) and action potential amplitude in the ventricular myocyte. The effect of \([K^{+}]_o\) in ventricular myocytes is abolished by \(Ba^{2+}\), suggesting that it is due to an increase in \(g_k\).

Before discussing the implications of our findings, it is important to consider the potential technical limitations of our voltage-clamp measurements.

Recordings of \(I_{Na}\) in cultured atrial myocytes were performed by using the amphotericin perforated-patch technique. We used this method because previous studies from our laboratory have shown that \(I_{Na}\) can be recorded without the potentially confounding influence of time-dependent changes in gating kinetics encountered with the conventional whole-cell configuration. \(I_{Na}\) was determined in solutions containing 150 mmol/L \(Na^{+}\) to simulate the normal physiological extracellular milieu and avoid the use of \(Na^{+}\) substitutes, which may themselves alter \(Na^{+}\) channel gating. In an effort to ensure adequate voltage control in these experiments, strict criteria were applied before data were accepted. These criteria were (1) a voltage error due to uncompensated series resistance <3.5 mV, (2) a negative limb of the I-V relation spanning at least 30 mV and showing no threshold phenomenon near the activation voltage of \(I_{Na}\), and (3) no crossover of current tracings as the magnitude of \(I_{Na}\) was decreased during the determination of steady-state inactivation. Using these indirect criteria, we believe that good control of membrane voltage was achieved during experiments with the perforated-patch technique. An additional criterion suggested by Hanck and Sheets\(^{41}\) is an activation curve slope factor >6.0 mV. In our experiments, the slope factor averaged 8.7±1.3 mV (n=7) under control conditions.

It is not possible to establish adequate voltage control during \(I_{Na}\) recording in ventricular cells or freshly isolated atrial cells when using a single-electrode whole-cell voltage clamp if solutions containing physiological \([Na^{+}]_o\) are used. We used excised outside-out patches to ensure voltage control in 150 mmol/L \([Na^{+}]_o\). Limitations
of this technique are (1) that patch excision causes a rapid negative shift in the voltage dependence of inactivation and (2) that this configuration, in our hands, was less stable than either perforated-patch or whole-cell recordings. In an attempt to minimize these problems, an abbreviated I-V protocol was used, and data were collected a minimum of 5 minutes after patch excision, since the largest and most rapid shifts in kinetics occur before that period.20 Furthermore, only experiments in which an I-V curve was obtained after washout of the elevated [K+]o were accepted for analysis. Thus, although we cannot exclude the possibility of small changes in activation or inactivation parameters in ventricular patches exposed to elevated [K+]o, such changes would not be of sufficient magnitude to explain the marked voltage-independent depression of V max observed in the ventricular cells.

Comparison With Other Studies of [K+]o and INa

To our knowledge, this is the first study to systematically examine the direct effects of [K+]o on INa in isolated cardiac myocytes. Dudel et al.25 while examining the steady-state background I-V relation and its [K+]o sensitivity in shortened Purkinje fibers, noted that a small Na+ current observed in 2.8 mmol/L [K+]o, during a constant-speed (300 ms V) depolarizing voltage ramp disappeared when [K+]o was raised to 10.8 mmol/L. These authors suggested that elevation of [K+]o may depress the excitatory Na+ system. Our results using isolated myocytes are in variance with this study. Reasons for the disparity between the two studies probably include differences in the voltage-clamp technique, the stimulus protocols (ramp versus step), and the adequacy of membrane voltage control.

The insensitivity of the cardiac Na+ channel to [K+]o shown in the present study contrasts with the reported behavior of the Na+ channel in neuronal preparations. In the squid giant axon, elevation of [K+]o from 5 to 25 mmol/L in the presence of a constant [Na+]o, (230 mmol/L) (1) shifts the steady-state inactivation curve in the hyperpolarized direction, (2) decreases the slope factor of the inactivation curve, (3) slows the time constant of recovery from inactivation, (4) decreases the peak INa amplitude in inverse proportion to log [K+]o, and (5) enhances the process of slow inactivation.21-23 In a study using voltage-clamped fibers from the frog node of Ranvier, Peganov et al.24 confirmed the enhancing effect of [K+]o on both the fast and slow processes of Na+ current inactivation. An increase in [K+]o from 2.5 to 25 mmol/L decreased the time constant for the development of slow inactivation by 50%, decreased the proportion of Na+ channels remaining free from slow inactivation from 0.8 to 0.45, and shifted the steady-state inactivation curve to the left along the voltage axis. We found no effect of 24 mmol/L [K+]o on the processes of fast or slow inactivation in atrial myocytes (Fig 2). This is in agreement with a study in guinea pig papillary muscle in which slow inactivation, determined by V max measurements, was unaffected by elevation of [K+]o to 10 mmol/L.42 Our results suggest that responsiveness to changes in [K+]o may represent a fundamental functional difference between the neuronal and cardiac subtypes of Na+ channels in addition to their differing sensitivity to tetrodotoxin43 and divalent cations.44,45 In the present study, [K+]o was confined to the range encountered during ischemia. Therefore, we cannot exclude an effect on the kinetics of INa at higher [K+]o (eg, 50 to 100 mmol/L).

Voltage-Independent Effect of [K+]o on V max

In atrial myocytes, 24 mmol/L [K+]o, caused no voltage-independent depression of V max. This was in marked contrast to the depression of V max observed in ventricular myocytes exposed to 14 mmol/L [K+]o. The depressant effect in ventricular myocytes could not be ascribed to a difference in the magnitude of INa applied during the action potential upstroke at the two [K+]o levels (see Fig 6). It was abolished by Ba2+, suggesting that it is due to an increase in gK predominantly carried through the inwardly rectifying K+ channel, IK.

Our findings in isolated ventricular cells are in qualitative agreement with the study of Kishida et al.20 in multicellular ventricular preparations. However, there are significant quantitative differences. We observed a 66% voltage-independent decrease in V max at 14 mmol/L [K+]o, compared with the 24.3% and 43% decreases reported in guinea pig papillary muscle exposed to 13.0 and 16.2 mmol/L [K+]o, respectively. These quantitative differences may reflect differences in experimental temperature and holding potential. Our experiments were performed at a lower temperature (20°C to 22°C versus 35°C) and at a more depolarized holding potential (−70 versus −83 mV) than those of Kishida et al. These conditions would be expected to slow the kinetics and increase the extent of steady-state inactivation of the Na+ channel and increase the amplitude of the background gK in both normal and elevated [K+]o. Thus, the V max value that reflects the maximal net inward membrane current is likely to be more sensitive to K2+-induced changes in gK at −70 mV, where the ratio of background Na+ conductance to gK is smaller than at −83 mV. Finally, significant interspecies variation in the shape and [K+]o sensitivity of the background I-V relation in the rabbit and guinea pig may also contribute to differences between the two studies. Differences in experimental preparations and conditions may also explain our findings of a significant (19%) voltage-independent depression of V max at 9 mmol/L [K+]o, whereas studies in guinea pig papillary muscle have found no effect at [K+]o<10.0 mmol/L.20,37 In contrast to the present findings, Weidmann,16 using voltage-clamped sheep Purkinje fibers, found no voltage-independent effect at a holding potential of −92 mV as [K+]o was raised to 13.5 mmol/L. The reasons for the discrepancy between the two studies are unclear but may again relate, in part, to differences in experimental temperature and holding potential.

Mechanisms of Voltage-Independent Effects of [K+]o in Ventricular Myocytes

Any proposed mechanism to explain the voltage-independent effects on V max observed in the present study must account for (1) its sensitivity to [K+]o, (2) the presence of an effect in ventricular but not atrial cells, and (3) abolition of the effect with Ba2+. Given these considerations, IK is likely to be the major conductance involved. The conductance of IK is very sensitive to [K+]o, (gK×V[K+]o) and is completely blocked by 1 mmol/L [Ba2+].46 Furthermore, compared with the large IK in ventricular cells, this current is very small in atrial
cells, in which no voltage-independent effect of [K+]o is seen. Other outward conductances, such as the transient outward current (Iₒ), are unlikely to contribute despite being sensitive to [K+]o. Under experimental conditions similar to the present study, Iₒ activates too slowly to influence phase 0 and is not completely abolished by 1 mmol/L Ba2+. Furthermore, Iₒ in the rabbit is 4 to 10 times larger in atrial than in ventricular cells, and yet no significant voltage-independent effect of [K+]o on Vmax is seen in atrial cells.36

How can an increase in IK1 produce a marked decrease in Vmax? At Vmax, the inward INa is the major conductance, with IK1 being <2% of INa. The marked decrease in Vmax must therefore predominantly reflect a decrease in the amplitude of INa. The voltage-clamp experiments suggest that under steady-state voltage conditions [K+]p, does not directly influence the amplitude or kinetics of INa. [K+]o must therefore exert its influence on INa via an indirect mechanism or mechanisms. The theoretical simulations using the Luo-Rudy model40 predict the same qualitative behavior as observed in the experiments and suggest one possible indirect mechanism for the effect of [K+]o on Vmax. The action potential upstroke is influenced by the history of membrane voltage before the onset of regenerative depolarization.47,48 Important determinants during this phase include the latency between the stimulus and the onset of the upstroke and the amplitude of the passive membrane-voltage response to the excitatory stimulus (the step potential). In the model simulations, a constant latency was maintained in 5 and 14 mmol/L [K+]o. To achieve an equivalent latency in high [K+]p requires a significantly larger excitatory stimulus, which, in turn, steps the membrane voltage to a more depolarized potential than under control conditions. This results in greater inactivation of INa during the stimulus pulse brought about by the fast inactivation h-gate with a much smaller contribution from the slow inactivation j-gate. Parameter m is not affected by the [K+]o changes. It is important to note that although an increase in the total outward K+ current is computed at elevated [K+]o (reflecting an increased conductance), its contribution to the decrease in Vmax is very small compared with a major effect due to greater inactivation of INa. This mechanism may contribute to our experimental findings, since the peak step potential was usually higher in 14 than in 5 mmol/L [K+]o, when action potentials of similar latency were compared (see Fig 5). Moreover, in the presence of Ba2+, which abolished the [K+]o-induced depression of Vmax, there were no significant differences in step potentials recorded at the two [K+]o levels (see Fig 9).

Although greater INa inactivation during excitation may partially explain our experimental findings in 14 mmol/L [K+]o, the quantitative difference (14% decrease of Vmax in the simulations, 66% in the experiments) suggests that other factors may be involved. One such factor may be species differences in membrane ionic currents (the model is a guinea pig type, whereas rabbit myocytes were used in the experiments). Perhaps a more significant factor, however, may relate to the greater complexity of the experimental preparation compared with the computer model. The Luo-Rudy model40 assumes uniform depolarization of a patch of membrane—a membrane action potential. Our experiments in ventricular cells, however, involve stimulation of an elongated three-dimensional structure via a point source of current. Under these conditions, the membrane is unlikely to be uniformly depolarized during excitation; thus, the action potential may reflect an element of propagation within the length of a single cell.39 If this assumption is correct, the voltage-independent effect of [K+]o on Vmax may reflect the influence of an increased outward “leak” via IK1. During normal excitation, IK1 is the source of inward current required to discharge the membrane capacitance. This initiates local circuit currents, which bring the membrane voltage to threshold in downstream membrane segments and enable propagation of the action potential along the cell. When IK1 is increased by elevating [K+]o, a greater component of the longitudinal current is shunted to the extracellular space and is therefore unavailable to discharge the downstream Ca++, thereby decreasing Vmax. This hypothesis is consistent with the notion that increased IK1 is the underlying mechanism responsible for the voltage-independent effect of [K+]o on Vmax. It may also explain the absence of such an effect in the cultured atrial cells, which, because of their spherical shape, small diameter, and minimal IK1, have significantly less resting outward “leak” and thus more uniform membrane depolarization during the action potential. Finally, abolition of the effects of [K+]o on ventricular Vmax with Ba2+ could also be attributed to inhibition of IK1, leading to an increase in Rm, improved spread of excitatory current from the point of stimulation, and thus a more uniform membrane action potential.

Conclusions

In summary, the present study has demonstrated that elevation of [K+]o within the range encountered in vivo during ischemia does not directly influence the kinetics of INa. Despite this, elevated [K+]o can influence the ventricular action potential upstroke via voltage-independent mechanisms, which involve an increase in the conductance of IK1. It seems likely that during ischemia, in addition to the depression of Vmax caused by membrane depolarization, voltage-independent depression of Vmax by [K+]o may contribute to the conduction slowing, which underlies the genesis of reentrant tachyarrhythmias. In this regard, it is interesting to note that the decrease in Vmax observed during experimental ischemia has been reported to exceed that expected from membrane depolarization alone.1 The voltage-independent effect of [K+]o may, together with such factors as extracellular acidosis and an increase in [Ca2+]i, augment this voltage-independent decrease in Vmax during ischemia.1

Acknowledgments

This study was supported in part by grants HL-32708, HL-32994, and HL-17676 from the National Institutes of Health. Dr Whalley is an Overseas Research Scholar of the National Heart Foundation of Australia. We wish to express our appreciation to Randy Rasmusson for his helpful comments and review of the final manuscript.

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_Circ Res._ 1994;75:491-502
doi: 10.1161/01.RES.75.3.491

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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