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Effects of Hypothermia, Potassium, and Verapamil on the Action Potential Characteristics of Canine Cardiac Purkinje Fibers

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Background: **Hypothermia may induce hypokalemia and increase intracellular Ca^{2+} by affecting serum K^+ and Ca^{2+} fluxes across the cell membrane. These ionic alterations may significantly change the electrophysiologic characteristics of the cardiac action potential and may induce cardiac arrhythmias. The current study was undertaken to determine whether electrophysiologic changes in Purkinje fibers induced by hypothermia could be reversed by manipulating the extracellular K^+ and transmembrane Ca^{2+} fluxes by Ca^{2+} channel blockade with verapamil.**

Methods: **A conventional microelectrode method was used to determine the effects of hypothermia (32 ± 0.5°C and 28 ± 0.5°C) and various external K^+ concentrations ($[K^+]_o$) (2.3, 3.8, and 6.8 mM) on maximum diastolic potential, maximum rate of phase 0 depolarization (V_{max}), and action potential duration (APD) at 50% (APD_{50}) and at 95% (APD_{95}) repolarization in isolated canine cardiac Purkinje fibers. To evaluate the contribution of the slow inward Ca^{2+} current to action potential changes in hypothermia, the experiments were repeated in the presence of the Ca^{2+}-channel antagonist verapamil (1 μM).**

Results: **Variations of $[K^+]_o$ induced the expected shifts in maximum diastolic potential, and hypothermia (28°C) induced moderate depolarization, but only when $[K^+]_o$ was ≥3.9 mM ($P < 0.05$). Hypothermia decreased V_{max} at all $[K^+]_o$ studied ($P < 0.05$). Regardless of the temperature, V_{max} was not affected by verapamil when $[K^+]_o$ was ≤3.9 mM, but at 6.8 mM $[K^+]_o$ in hypothermia V_{max} was significantly lower in the presence of verapamil. Hypothermia increased both the APD_{50} and the APD_{95}. The effects of verapamil on APD were temperature and $[K^+]_o$ dependent; between 37°C and 28°C with 2.3 mM $[K^+]_o$ in the superfusate, verapamil did not affect APD. At 28°C in the presence of verapamil, the APD_{50} and APD_{95} decreased only if the $[K^+]_o$ was ≥3.9 mM.**

Conclusions: **Verapamil and K^+ supplementation in hypothermia may exert an antiarrhythmic effect, primarily by reducing the dispersion of prolonged APD. (Key words: Calcium channel, antagonist: verapamil. Heart, electrophysiology: action potential duration; maximum diastolic potential; maximum rate of phase 0 depolarization. Ions, potassium: extracellular. Temperature: hypothermia.)**

HYPOTHERMIA can occur accidentally after exposure to a cold environment or operating room or may be intentionally induced to protect tissues against hypoxia during surgery.[1] Various cardiac arrhythmias, including ventricular fibrillation and asystole, occur at temperatures below about 30°C.[2] An anesthesiologist may be faced with this problem in the critical care setting while treating the patient with accidental hypothermia, intraoperatively during recovery from low temperature cardioplegia, or even during irrigation of the thoracic cavity with cold solution.[3] Major electrocardiographic manifestations of hypothermia include decreased myocardial conduction velocity with increases in PR and QT intervals and QRS complex duration.[2] At least in part, these changes can be attributed to alterations in the electrophysiologic properties of the cardiac Purkinje fibers[4] or serum electrolyte changes[5] induced by the hypothermia.[6,7] *In vivo*, nonhomogeneous tissue cooling slows action potential propagation and can induce differences in repolarization at various sites in the ventricular conducting system.[8] This repolarization

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heterogeneity facilitates nonuniform distribution of impulse conduction throughout the myocardium and may result in reentrant arrhythmias.[8–11] In addition, low temperature modifies cation conductance across cell membranes[12] and may result in clinically significant hypokalemia, which can be attributed to a temperature-mediated redistribution of K^+ among body compartments.[6,7] The resting membrane potential of cardiac Purkinje fibers may depolarize in deep hypothermia,[13] and an increase in the action potential duration (APD) is seen even during mild hypothermia and hypokalemia.[14] These electrophysiologic changes can depress impulse conduction and may induce reentry arrhythmias, abnormal forms of automaticity, and heart block.[5]

The exact mechanism by which hypothermia increases cardiac irritability is unknown, but the hypothermia-induced electrophysiologic changes in Purkinje fiber action potentials may be involved. In addition to affecting K^+ homeostasis,[6,7] hypothermia also increases intracellular myocardial Ca^{2+} concentration ($[Ca^{2+}]_i$).[15] Inhibition of the Na^+–K^+ pump activity by low temperature leads to an increase in intracellular Na^+ concentration ($[Na^+]_i$), which may promote Ca^{2+} influx during the plateau of the action potential or oppose a Ca^{2+} efflux during diastole by the Na^+–Ca^{2+} exchange mechanism.[16] The excessive accumulation of intracellular Ca^{2+}, termed "Ca^{2+} overload,"[17] may result in delayed afterdepolarizations, causing severe cardiac arrhythmias.[5,17–19] Furthermore, it has been suggested that hypothermia, by delaying inactivation of the inward Ca^{2+} current (I_{Ca}) and maintaining the I_{Ca} for a longer time, contributes to action potential lengthening and arrhythmias based on abnormal impulse propagation.[20]

We hypothesized that if increased $[Ca^{2+}]_i$ underlay important changes in electrophysiologic characteristics of Purkinje fibers in hypothermia, especially prolongation of the APD, we should be able to alter their course with a Ca^{2+}-channel blocking agent. Because hypothermia alters K^+ homeostasis,[6,7] we also examined the effects of hypothermia on action potential characteristics over the wide range of external $[K^+]$ ($[K^+]_o$) in the absence and presence of verapamil. The reduction of APD with verapamil, if present, might be expected to reduce the incidence of hypothermia-induced arrhythmias, specifically dysrhythmias based on regional differences in myocardial repolarization.

Materials and Methods

This study was approved by the Medical College of Wisconsin Animal Care Committee and conformed with standards set forth in the *Guide for Care and Use of Laboratory Animals*.#

Adult mongrel dogs (10–22 kg, of either sex) (n = 45) were anesthetized with 30 mg/kg pentobarbital sodium. The heart was quickly excised, and the anterior false tendon with attached papillary muscle from the left ventricle was removed and immersed in modified Krebs' solution (22°C) equilibrated with 97% O_2 and 3% CO_2. Small (<1-cm^2) preparations with free-running strands of Purkinje fibers were dissected from this tissue and pinned to the silicone elastomere floor of a 2-ml chamber and superfused at a rate of 4 ml/min with modified Krebs' solution (37°C) containing 2.3, 3.9, or 6.8 mM KCl with or without 1 μM verapamil and equilibrated with a 97% O_2–3% CO_2 gas mixture. The millimolar composition of Krebs' solution was 137 NaCl, 12 $NaHCO_3$, 1.8 NaH_2PO_4, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 5.5 glucose, and 0.05 EDTA, with a *p*H of 7.4.

Each preparation was stimulated at a constant rate (1 Hz) with the use of bipolar-silver wire endocardial surface electrodes. The stimuli were square-wave pulses lasting 2 ms at 1.5 times threshold. Transmembrane action potentials were recorded with conventional microelectrode techniques. Action potential changes stabilized within 5–8 min. Glass microelectrodes (15–30-MΩ resistance) were coupled by Ag–AgCl wire to a preamplifier (World Precision Instruments, New Haven, CT). Action potential signals were recorded on frequency-modulated tape (AR Vetter, Rebersburg, PA) for later analysis of maximum diastolic potential (MDP), maximum rate of phase 0 depolarization (V_{max}), and APD at 50% (APD_{50}) and at 95% (APD_{95}) repolarization. These values were displayed and measured electronically directly off the digital oscilloscope (Nicolet 310). The V_{max} was determined with a differentiator exhibiting a linear response from 100–1,000 V/s. The "zero" potential was obtained at the beginning and at the end of the experiments by withdrawing the microelectrode from the inside of the fiber. Because the ground connection between the bath and the circuit was made through a direct Ag–AgCl connection, the change in temperature may slightly influence the half cell potential in the bath, and this change will sum

Guide for Care and Use of Laboratory Animals. Publication 85-23. Bethesda, MD, Public Health Services, National Institutes of Health, revised 1985.

with the real MDP changes. To verify the significance of this phenomenon we performed additional experiments (n = 8) in which the microelectrode was placed into the bath, the temperature was changed from 37°C to 28°C, and the Ag–AgCl bath junction potential change was measured. The half cell potential caused a hyperpolarization to be measured by the microelectrode over this temperature range by −2.1 ± 0.02 mV and −4.2 ± 0.05 mV at 32°C and 28°C respectively. All measured MDP values at 32°C and 28°C were corrected for the above differences, by subtracting the temperature-induced bath potential from the actual measured potential, and all the statistical analyses were performed with these corrected values.

The tissue bath was surrounded by a thermostatically controlled water bath, maintained at a constant temperature of 37 ± 0.02°C. The low bath superfusate temperatures (32 and 28 ± 0.5°C) were attained by readjusting the setting of the thermostat. The temperature in the tissue bath was gradually decreased from 37°C to 25°C over a 20–30-min period. The temperature of the solution was measured by a small, rapidly responding, custom made thermistor probe placed less than 2 mm from the preparation. The fluid level in the chamber was kept at a constant height (4 mm) by continuous suction.

The preparations were allowed to equilibrate for about 1 h. To determine the effects of hypothermia on action potential characteristics, action potentials were recorded at 37°C, 32°C, and 28°C (±0.5°C). To determine the effects of various $[K^+]_o$ on action potentials, experiments were performed with 2.3, 3.9, and 6.8 mM $[K^+]$ in the superfusate. Verapamil (Sigma, St. Louis, MO) prepared as stock solution (100 μM) was added to measured volumes to achieve the desired 1 μM concentration in the superfusate. The same set of action potential measurements were performed with 1 μM verapamil in the superfusate. Tissues were exposed to each $[K^+]_o$ and to verapamil (1 μM) for 20 min before measurement of action potential characteristics.

Data are expressed as means ± standard error of the mean. Statistical analysis was performed by paired and unpaired *t* tests and with one-way analysis of variance (analysis of variance repeated measures and factorial analysis), as appropriate, with $P < 0.05$ considered statistically significant.

Results

Typical effects of hypothermia on the action potential in canine Purkinje fibers are illustrated in the upper panel of figure 1. Each preparation was stimulated at a constant rate of 1 Hz. Recordings were taken from the same cell at various temperatures at a $[K^+]_o$ of 3.9 mM. Hypothermia significantly increased APD. The bottom panel of figure 1 shows the changes of action potential in hypothermia in the presence of 1 μM verapamil in the superfusate. In the presence of verapamil, the Purkinje fiber's action potential is shorter than at the same temperature without the drug (dashed action potential was recorded at 37°C in the absence of verapamil). The slope of phase 2 repolarization is increased by verapamil, a finding that is consistent with the blockade of a slow inward current such as that carried by a Ca^{2+} ion. Hypothermia, on the other hand, appears to decrease the slope of phase 2, suggesting that low temperature affects the Ca^{2+}-dependent ionic mechanisms in opposite direction.

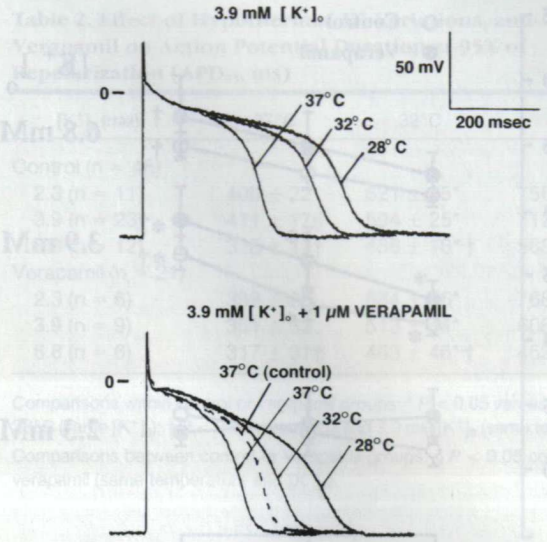

Fig. 1. Effect of hypothermia on action potential of canine cardiac Purkinje fiber superfused with normal Krebs' solution (*top*) and in the presence of verapamil (*bottom*). See text for details.

Maximum Diastolic Potential

The effects of two stages of hypothermia (32°C and 28°C) on the MDPs of Purkinje fibers were recorded during the exposure to low (2.3 mM), normal (3.9 mM), and high (6.8 mM) $[K^+]_o$ in the superfusate (fig. 2). MDPs were higher (hyperpolarized) at low, and lower (depolarized) at high $[K^+]_o$ than at normal $[K^+]_o$ ($P < 0.05$), regardless of the temperature. During the 20–30 min of gradual cooling from 37°C to 28°C, MDP

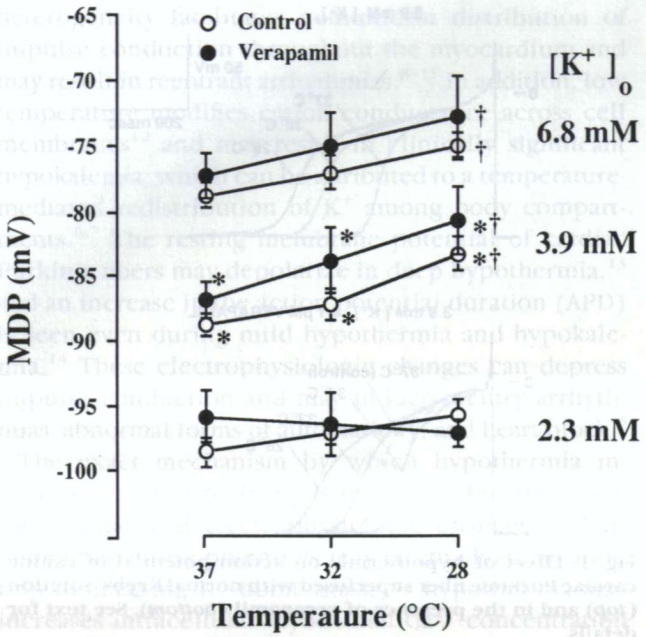

Fig. 2. Effect of hypothermia and K^+ variations on maximum diastolic potential (MDP) of Purkinje fiber action potentials with and without verapamil in the superfusate. *$P < 0.05$ *versus* 2.3 and 6.8 mM external $[K^+]$ ($[K^+]_o$) at same temperature. †$P < 0.05$ *versus* 37°C at same $[K^+]_o$.

decreased at normal and high $[K^+]_o$ ($P < 0.05$), and did not change at low $[K^+]_o$.

At 37°C and in the presence of verapamil MDP was lower (difference not significant, $P > 0.05$ *vs.* control; fig. 2). In hypothermia and at $[K^+]_o \geq 3.9$ mM loss of membrane potential was parallel to this of control Purkinje fibers, and reached significant depolarization at 28°C ($P < 0.05$). At $[K^+]_o$ 2.3 mM there was no effect of temperature on MDP in verapamil-superfused Purkinje fibers.

Maximum Rate of Phase 0 Depolarization

The effects of hypothermia, K^+ variation, and verapamil on V_{max} are summarized in figure 3. Hypothermia decreased V_{max} at each $[K^+]_o$ ($P < 0.05$). Despite significant differences in MDP at various $[K^+]_o$ at 37°C (fig. 2), the respective V_{max} values were not different (fig. 3), although there were trends for V_{max} to be lower at 6.8 and higher at 2.3 mM $[K^+]_o$ than at 3.9 mM $[K^+]_o$ ($P > 0.05$). At $[K^+]_o \leq 3.9$ mM with verapamil in the superfusate no additional effect, besides that of temperature, was noted on V_{max} (fig. 3). At 6.8 mM $[K^+]_o$ in hypothermia, V_{max} was lower in the presence of verapamil ($P < 0.05$).

To quantify the effects of changing MDP on V_{max}, we examined eight additional Purkinje fiber preparations by gradually increasing $[K^+]_o$ in the superfusate from 0.8 to 10 mM; MDP and V_{max} values were measured at 37°C and at 30°C (fig. 4). At both temperatures MDP decreased (less negative) directly with increasing $[K^+]_o$ between 2.3 and 10 mM ($P < 0.05$); below 2.3 mM MDP did not further change ($P > 0.05$). Only between 3.9 and 8.5 mM $[K^+]_o$ MDP was lower at 30°C ($P < 0.05$). V_{max} decreased moderately between 2.3 and 6.8 mM $[K^+]_o$, followed by a rapid decrease when $[K^+]_o$ increased above 6.8 mM (MDP ≤ −80 mV) (fig. 4). Compared with 2.3 mM $[K^+]_o$, at 0.8 mM $[K^+]_o$ there was a

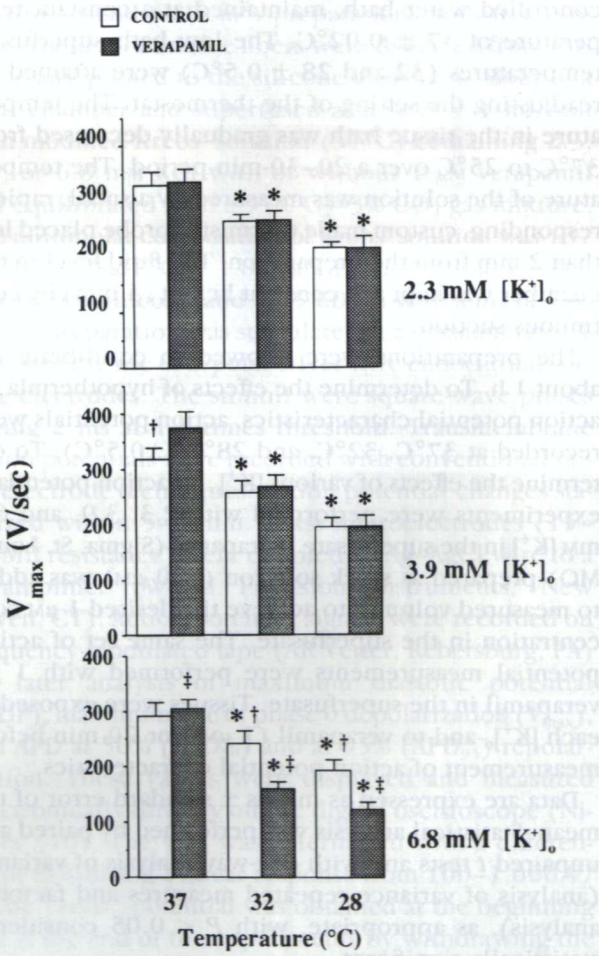

Fig. 3. Effect of hypothermia and K^+ variations on the maximum rate of phase 0 depolarization (V_{max}) of Purkinje fiber action potentials with and without verapamil in the superfusate. *$P < 0.05$ *versus* 37, 32, and 28°C; †$P < 0.05$ *versus* verapamil; ‡$P < 0.05$ *versus* 2.3 and 3.9 mM external $[K^+]$ + verapamil.

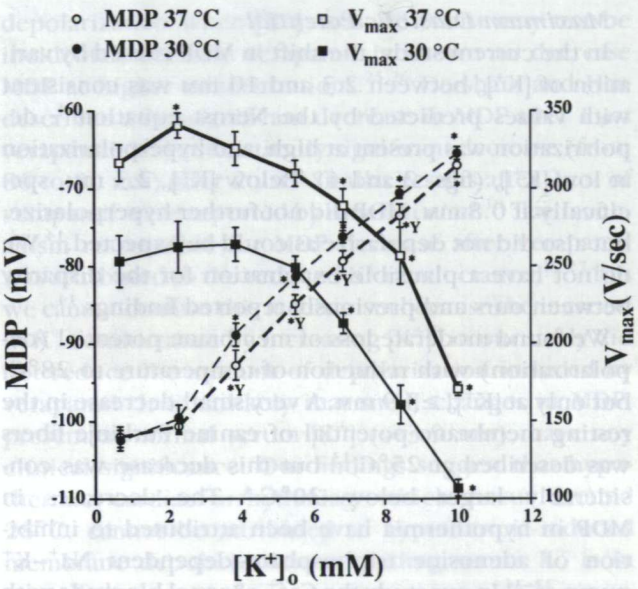

Fig. 4. Relation between maximum diastolic potential (MDP) and maximum rate of phase 0 depolarization (V_{max}) of Purkinje fiber action potentials at 37°C and at 30°C during the gradual increase in external $[K^+]$ ($[K^+]_o$) from 0.8 to 10 mM. *$P < 0.05$ MDP or V_{max} *versus* lower $[K^+]_o$. $^Y P < 0.05$ $MDP_{37°C}$ *versus* $MDP_{30°C}$ at same $[K^+]_o$.

significant decrease in V_{max} at 37°C, and no change in V_{max} at 30°C. By varying the $[K^+]_o$, V_{max} at 37°C and 30°C resulted in similar pattern of changes, yet amplitudes were different.

Action Potential Duration

Hypothermia increased early (APD_{50}) and late (APD_{95}) stages of repolarization (tables 1 and 2). Figures 5 and 6 compare the relative changes in APD_{50} and APD_{95} at various temperatures and $[K^+]_o$ with and without verapamil in the superfusate. In hypothermia the increase in APD_{50} (at 28°C) and APD_{95} (at 32°C and 28°C) was inversely related to $[K^+]_o$; at 6.8 mM $[K^+]_o$ in hypothermia APD_{50} and APD_{95} were always closest to normothermic control. Regardless of temperature, verapamil did not affect APD_{50} or APD_{95} at 2.3 mM $[K^+]_o$. At 28°C and $[K^+]_o \geq 3.9$ mM verapamil significantly shortened the APD_{50} (fig. 5) and the APD_{95} (fig. 6). At 28°C and 6.8 mM $[K^+]_o$ with verapamil, the

Table 1. Effect of Hypothermia, K^+ Variations, and Verapamil on Action Potential Duration at 50% of Repolarization (APD_{50}, ms)

$[K^+]_o$ (mM)	37°C	32°C	28°C
Control (n = 46)			
2.3 (n = 11)	216 ± 12	321 ± 21*	393 ± 27*
3.9 (n = 23)	218 ± 9	314 ± 14*	367 ± 17*§
6.8 (n = 12)	166 ± 7†	235 ± 10*†	297 ± 12*†§
Verapamil (n = 21)			
2.3 (n = 6)	213 ± 18	334 ± 30*	376 ± 16*
3.9 (n = 9)	194 ± 16	287 ± 21*	293 ± 20*‡
6.8 (n = 6)	139 ± 9†	187 ± 7*†	172 ± 16*†

Comparisons within control or verapamil groups: * $P < 0.05$ *versus* 37, 32, and 28°C (same $[K^+]_o$); † $P < 0.05$ *versus* 2.3 and 3.9 mM $[K^+]_o$ (same temperature); ‡ $P < 0.05$ *versus* 2.3 or 6.8 mM $[K^+]_o$ (same temperature).

Comparisons between control or verapamil groups: § $P < 0.05$ control *versus* verapamil (same temperature and $[K^+]_o$).

Table 2. Effect of Hypothermia, K^+ Variations, and Verapamil on Action Potential Duration at 95% of Repolarization (APD_{95}, ms)

$[K^+]_o$ (mM)	37°C	32°C	28°C
Control (n = 46)			
2.3 (n = 11)	409 ± 22	621 ± 35*	756 ± 42*
3.9 (n = 23)	411 ± 17§	594 ± 25*	712 ± 27*§
6.8 (n = 12)	328 ± 12†	456 ± 16*†	563 ± 24*†§
Verapamil (n = 21)			
2.3 (n = 6)	399 ± 28	584 ± 46*	768 ± 49*
3.9 (n = 9)	351 ± 32	513 ± 44*	606 ± 60*
6.8 (n = 6)	317 ± 31†	463 ± 46*†	462 ± 45*†

Comparisons within control or verapamil groups: * $P < 0.05$ *versus* 37, 32, and 28°C (same $[K^+]_o$); † $P < 0.05$ *versus* 2.3 and 3.9 mM $[K^+]_o$ (same temperature).

Comparisons between control or verapamil groups: § $P < 0.05$ control *versus* verapamil (same temperature and $[K^+]_o$).

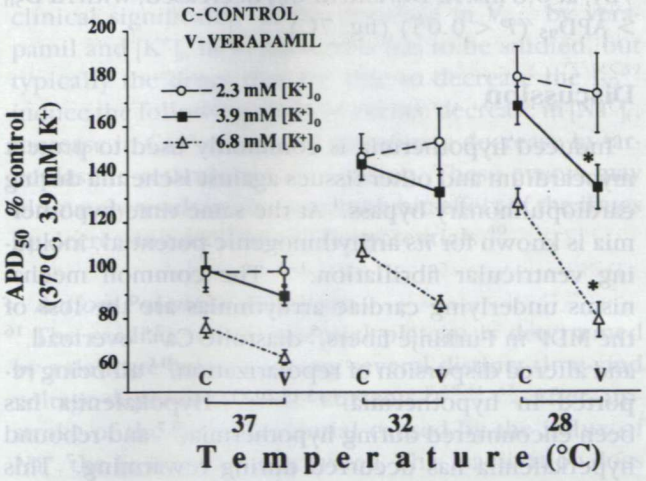

Fig. 5. Relative changes in action potential duration at 50% repolarization (APD_{50}) by verapamil at various external $[K^+]$ ($[K^+]_o$) during cooling (control [C] values are normalized to the values obtained at 37°C and at 3.9 mM $[K^+]_o$). *$P < 0.05$ V (verapamil) *versus* C (control) at respective temperature.

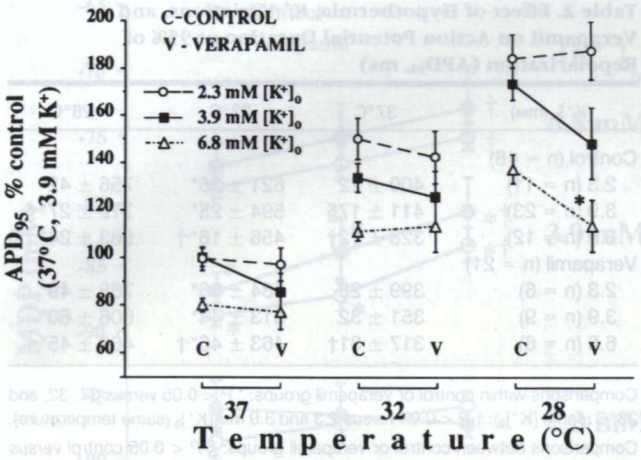

Fig. 6. Relative changes in action potential duration at 95% repolarization (APD_{95}) by verapamil at various external $[K^+]$ ($[K^+]_o$) during cooling (control [C] values are normalized to the values obtained at 37°C and at 3.9 mM $[K^+]_o$). *$P < 0.05$ V (verapamil) *versus* C (control) at respective temperature.

APD_{50} was 21% shorter whereas the APD_{95} was 12% longer than normothermic control. Without the verapamil at 28°C, there were no relative differences between the increases in APD_{50} and APD_{95}, regardless of the $[K^+]_o$, indicating that hypothermia prolonged early and late repolarization similarly (fig. 7). After verapamil was introduced to the superfusate, the relative changes in APD became dependent on the actual $[K^+]_o$; at 2.3 mM $[K^+]_o$ APD_{50} and APD_{95} were unchanged (fig. 7A); at 3.9 mM $[K^+]_o$ the primary effect was on APD_{50} (fig. 7B); at 6.8 mM APD_{50} and APD_{95} decreased, with $APD_{50} > APD_{95}$ ($P < 0.05$) (fig. 7C).

Discussion

Induced hypothermia is commonly used to protect myocardium and other tissues against ischemia during cardiopulmonary bypass.[1] At the same time hypothermia is known for its arrhythmogenic potential, including ventricular fibrillation.[1–3] The common mechanisms underlying cardiac arrhythmias are the loss of the MDP in Purkinje fibers,[5] diastolic Ca^{2+} overload,[16] and altered dispersion of repolarization,[11] all being reported in hypothermia.[4,13,14,20,21] Hypokalemia has been encountered during hypothermia,[6,7] and rebound hyperkalemia has occurred during rewarming.[7] This study examined electrophysiologic alterations of MDP, V_{max}, and APD during hypothermia and hypothermic hypokalemia and their reversibility after verapamil treatment and K^+ supplementation.

Maximum Diastolic Potential

In the current study, the shift in MDP caused by variation of $[K^+]_o$ between 2.3 and 10 mM was consistent with values predicted by the Nernst equation[14]: depolarization was present at high, and hyperpolarization at low $[K^+]_o$ (figs. 2 and 4). Below $[K^+]_o$ 2.3 mM, specifically at 0.8 mM, MDP did not further hyperpolarize but also did not depolarize as could be expected.[14] We do not have a plausible explanation for the disparity between ours and previously reported findings.[14]

We found moderate loss of membrane potential (depolarization) with reduction of temperature to 28°C but only at $[K^+]_o \geq 3.9$ mM. A very small decrease in the resting membrane potential of canine Purkinje fibers was described at 25°C,[13] but this decrease was considerably larger below 20°C.[4] The decreases in MDP in hypothermia have been attributed to inhibition of adenosine triphosphate–dependent Na^+–K^+ pump.[22–24] In our study the Ca^{2+}-channel blockade with 1 μM verapamil had no significant additional effect on MDP regardless of the temperature, and when $[K^+]_o$ was ≥ 3.9 mM moderate depolarization at 28°C was parallel to that of control Purkinje fibers. This tendency toward

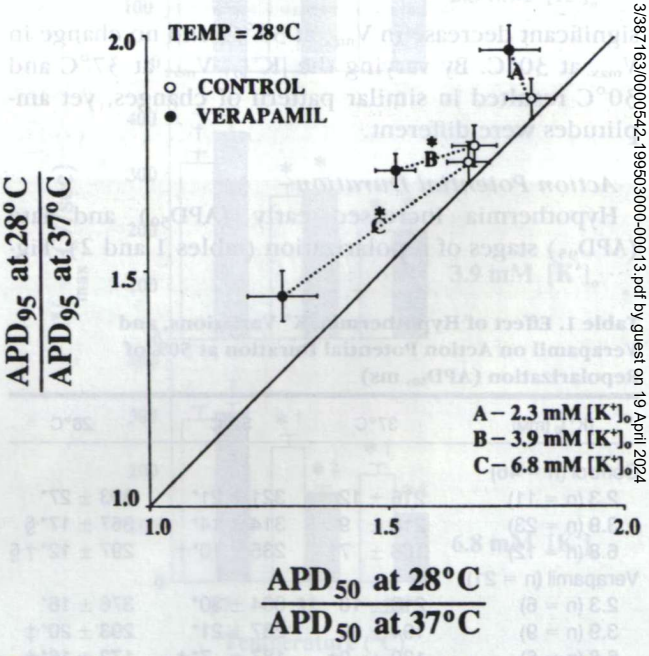

Fig. 7. Effect of verapamil (closed symbols) on the relative relation between action potential duration at 50% (APD_{50}) and 95% (APD_{95}) repolarization at 28°C at various external $[K^+]$ ($[K^+]_o$). Values are normalized to their respective controls at 37°C (open symbols). *$P < 0.05$ describes the significance of relative changes between action potential duration at 50% (APD_{50}) *versus* 95% (APD_{95}) repolarization after verapamil.

depolarization when $[K^+]_o$ was ≥3.9 mM may not be incidental, because verapamil is known to decrease MDP at higher concentrations.[25,26] Dersham and Han described a nonsignificant decrease in MDP after 1 μM verapamil, and a statistically significant decrease (from 86 to 80 mM) after 2 μM.[9] Similar results after 2 μM of verapamil were reported by Amerini *et al.*[19] The mechanism responsible for MDP decrease after verapamil was attributed to the reduced K^+ conductance,[19,27] but we cannot confirm this mechanism because the increase in K^+ conductance by increasing $[K^+]^8$ in our study did not reduce the amount of depolarization induced by verapamil. It is unlikely that small changes in MDP, present at normal serum $[K^+]$ (≈3.9 mM), have any clinical significance. Our findings suggest that hypothermic cardiac arrhythmias, which occur around 28°C, cannot be attributed to hypothermia-induced membrane depolarization, providing serum K^+ is in normal range.

Maximum Rate of Phase 0 Depolarization

The magnitude of V_{max} is determined by the fast inward Na^+ current (I_{Na}), and has traditionally been used as an index of Na^+ channel availability.[28,29] In our experiments, hypothermia decreased V_{max} (fig. 3), which may be explained by the temperature-mediated decrease in I_{Na}.[24,30] Hypothermia decreases Na^+–K^+ adenosine triphosphatase activity, which then results in increased $[Na^+]_i$ and decrease in driving force for I_{Na}.[31–33] At $[K^+]_o$ ≤3.9 mM in the normothermic and hypothermic states, Ca^{2+}-channel blockade with 1 μM verapamil did not alter V_{max}. Although verapamil in lower concentrations has no significant effect on V_{max},[9,25,34,35] the Ca^{2+}-channel blockade may decrease V_{max} when given in higher concentrations.[36,37] However, at 6.8 mM $[K^+]_o$ in hypothermia, we found that verapamil decreased V_{max} ($P < 0.05$) (fig. 3). It is possible that the cumulative effect of hypothermic I_{Na} blockade, low MDP caused by hyperkalemia, and verapamil-induced inhibition of I_{Na},[36,37] possibly potentiated by low temperature, resulted in a reduction of V_{max}.

Because V_{max} is voltage-dependent we examined the relation between MDP and V_{max} by varying the $[K^+]_o$ between 0.8 and 10 mM. Whereas the increase in $[K^+]_o$ between 2.3 and 10 mM caused an almost linear decrease in MDP (depolarization), at less than 2.3 mM, MDP ceased to decrease further, consistent with a decrease in resting K^+ conductance at low $[K^+]_o$.[38] The decrease in temperature from 37°C to 30°C induced a leftward shift of the V_{max} curve. Although there was a decreasing trend, V_{max} did not significantly change when $[K^+]_o$ was increased from 2.3 to approximately 6 mM, a finding that is consistent with the results described in figure 3. Therefore, V_{max} was not greatly affected when the voltage (MDP) was between −100 and −85 mV (2.3–6 mM $[K^+]_o$), but was markedly decreased when MDP became less negative than −80 mV. In extreme hypokalemia (0.8 mM) at 37°C V_{max}, significantly decreased, at least in part mirroring changes in the respective MDP. Our data suggest that during phase 0 of the stimulated action potential at MDP between −80 and −100 mV, the fraction of available Na^+ channels remains relatively constant or only slightly increases as MDP becomes more negative. Thus, when MDP reaches −80 mV, the near-maximum capacity for Na^+ influx through the fast Na^+ channels may be reached (especially in hypothermia) and cannot be further increased by more negative MDP. The decrease in V_{max} when MDP drops below approximately −72 mV at 37°C and −80 mV at 30°C, is very abrupt. In addition, hypothermia significantly decreased V_{max} and less affected MDP. These findings are consistent with the existence of two mechanisms that may effect Na^+-channel kinetics: first, a temperature-dependent mechanism that is responsible for the decrease in V_{max} during cooling, and second, a voltage-dependent mechanism that is active only at MDP less negative than −80 mV. In deep hypothermia and at $[K^+]_o$ 6.8 mM, verapamil further decreased V_{max}, most likely by the interference with I_{Na} kinetics by voltage-dependent mechanism. The clinical significance of the decrease in V_{max} by verapamil and $[K^+]_o$ in hypothermia has to be studied, but typically the drugs that are able to decrease the I_{Na}[39] induce the following chain of events: decrease in $[Na^+]_i$, increase in Ca^{2+} efflux and therefore a decrease in sarcoplasmic reticulum Ca^{2+} loading. These events may ultimately result in an antiarrhythmic effect of the drugs by decreasing oscillatory afterpotentials.[40]

Action Potential Duration

The cardiac action potential plateau is determined by a delicate balance among several distinct time- and voltage-dependent ionic currents.[8,38,41–43] After upstroke of the action potential caused by the influx of Na^+, the I_{Na} becomes inactivated. This inactivation does not lead to immediate repolarization, because the initial depolarization reduces the inwardly rectifying K^+ conductance and opens voltage-gated Ca^{2+} channels thus permitting the intracellular influx of Ca^{2+} by the I_{Ca}.

During the action potential plateau, while membrane conductance for all ions is reduced, several currents help to maintain the transmembrane potential at around 0 mV, including I_{Ca}, Na^+ "window," Cl^- and inward (anomalous) rectifying K^+ currents. In addition, currents produced by the electrogenic Na^+–K^+ pump and Na^+–Ca^{2+} exchange mechanism help to maintain the action potential plateau. The outward (delayed) rectifying K^+ current (I_K) and the inward rectifying K^+ current are responsible for final rapid repolarization (phase 3). Details of the ionic basis of action potential are reviewed elsewhere.[5,16,23,27,38,44,45]

The electrophysiologic mechanisms responsible for the marked prolongation of APD seen in hypothermia are not fully understood, although mechanisms based on temperature dependence of I_K[46] and I_{Ca}[47] have been proposed. One important mechanism may be the hypothermia-induced reduction of I_K, which is a major contributor to membrane repolarization.[46] In addition, lengthened APD in hypothermia may be attributed to delayed inactivation of the I_{Ca},[47,48] which will maintain the I_{Ca} for a longer time.[20] Finally, prolonged APD may be also attributed to increase in $[Ca^{2+}]_i$ through altered Na^+–Ca^{2+} exchange.[33] The activity of the Na^+–K^+ pump is likely to be reduced in hypothermia[31,32] resulting in a net rise in $[Na^+]_i$.[33] The increase in $[Na^+]_i$ may promote Ca^{2+} influx during the plateau of the action potential or reduce Ca^{2+} efflux during diastole by Na^+–Ca^{2+} exchange.[49] Although the activity of the Na^+–Ca^{2+} exchanger is temperature dependent, its relatively low Q_{10} (the rate of exchange produced by changing the temperature 10°C) means that this mechanism may contribute to raising diastolic free $[Ca^{2+}]_i$.[44]

We have demonstrated that hypothermia equally lengthens the APD_{50} and APD_{95}, indicating that low temperature similarly affects the ionic mechanisms that determine the early and late stages of repolarization (tables 1 and 2 and fig. 7), *i.e.* earlier discussed I_K and I_{Ca}. The finding that hypokalemia, similarly to hypothermia, prolongs the APD is not surprising, because low $[K^+]_o$ decreases K^+ conductance.[38] APD_{50} and APD_{95} were shorter at 6.8 mM $[K^+]_o$ than at either 3.9 or 2.3 mM $[K^+]_o$ (figs. 5 and 6) as a result of improved K^+ conductance.[8,40,45,50] Interventions that affect ionic currents during the repolarization phase may selectively alter the shape and duration of the cardiac action potential. Verapamil may affect both the I_{Ca} and K^+ conductance.[43,51] In our study, 1 μM of verapamil significantly shortened both the APD_{50} and the APD_{95}, but only at 28°C and $[K^+]_o \geq 3.9$ mM (figs. 5 and 6). At this temperature and with 6.8 mM $[K^+]_o$ and verapamil in the superfusate, the relative shortening of APD_{50} exceeded that of APD_{95} (fig. 7, C). The observation that earlier phases of repolarization (APD_{50}) appear to be more readily affected by verapamil is consistent with the primary blocking action of this agent on I_{Ca}. At the same time less affected shortening of APD_{95} by verapamil, and its significant shortening at higher $[K^+]_o$ suggests that another mechanism is involved in the lengthening of APD at low temperature, presumably hypothermia-induced reduction of I_K.[46] Also, when $[K^+]_o$ was sufficiently high at 28°C, both APD_{50} and APD_{95} were shortened, indicating not only an important role of $[K^+]_o$ in increasing K^+ conductance but also an interaction between Ca^{2+}-channel blockade and K^+ conductance. Our study indicates that hypokalemia must be corrected if the APD is to be shortened with verapamil. Not only does correction of hypokalemia affects APD through increases of K^+ conductance,[38] but in addition, as Cavalié *et al.* have shown, I_{Ca} inactivation (which is delayed by hypothermia) depends on voltage and is greater at a more depolarized potential,[52] as in our study at 6.8 mM.

Different arrhythmias may arise if the $[Ca^{2+}]_i$ is increased or if cardiac action potential is lengthened. First, increase in $[Ca^{2+}]_i$ caused by hypothermia results in oscillatory release of Ca^{2+} from sarcoplasmic reticulum; this may generate the transient inward current allowing more Na^+ and Ca^{2+} into the cell creating delayed afterdepolarization.[5,17,38] When delayed afterdepolarization reaches certain threshold, arrhythmias may be triggered. These arrhythmias can be seen at low $[K^+]_o$, and when Na^+ extrusion from the cell is reduced, because both may be encountered during hypothermia. By diminishing $[Ca^{2+}]_i$ influx, verapamil reduces Ca^{2+} overload, delays afterdepolarization amplitude and reduces triggered automaticity.[53,54] Verapamil thus may be useful for treatment of hypothermia-induced arrhythmias based on delayed afterdepolarization. Second, reentry arrhythmias may occur during the propagation of slow action potentials through Purkinje fibers[5,8,21,34,55] especially *in vivo,* when, because of uneven tissue cooling, heterogeneity of repolarizations may ensue.[8] A shortening of APD with verapamil in hypothermia is both temperature and $[K^+]_o$ dependent, and this may have significant clinical implications, but only at higher $[K^+]_o$: the lower the temperature, the longer the APD, and the greater the shortening effect of verapamil. Because the APD is very dependent on regional myocardial temperature, verapamil selectively

affects APD: a greater APD in a cooler region will be decreased more than a lesser APD in a warmer region. By reducing the regional differences in myocardial repolarization this effect may decrease the propensity for development of arrhythmogenic reentry circuits.

In conclusion, there appears to be an increased relative I_{Ca} at low temperature, as evidenced by the effective shortening of APD in deep hypothermia by verapamil. Although this shortening may result from an increase in I_{Ca}, a decrease in K^+ conductance at low temperature is more likely, as evidenced by the effective shortening of APD when the K^+ conductance is increased by increasing $[K^+]_o$. Finally, the effectiveness of verapamil to shorten APD in hypothermia was dependent on $[K^+]_o$ suggesting that the increase in K^+ conductance is an important factor for achieving this verapamil effect. Verapamil and K^+ supplementation in hypothermia may have an antiarrhythmic effect primarily by reducing the dispersion of prolonged APD. Further studies will be needed to evaluate this antiarrhythmic effect *in vivo*.

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