Mechanism of Low $K^+$-induced Depolarization in Mammalian Cardiac Muscle

Duck Sun Ahn, Syng Ill Lee and Doo Hee Kang

The membrane permeability to potassium at a resting state is greater than to any other ions and the maintenance of resting membrane potential is largely dependent on $K^+$ concentration of outside medium (Hodgkin and Horowicz 1959), i.e., an increase of $K^+$ concentration of medium induces a depolarization, vice versa. However, on the contrary to this prediction, in some mammalian heart muscle a reduction of external $K^+$ concentration induces a depolarization of membrane potential rather than a hyperpolarization (Vassalle 1965). In this study it was aimed to elucidate the possible mechanism of spontaneous depolarization induced by low external $K^+$ in canine Purkinje fibers. The membrane potential was constantly recorded while components of cations in the bathing medium were replaced one by one by equimolar sucrose until the low $K^+$ induced depolarization was blocked. The results are summarized as follows; The membrane potential of canine Purkinje fibers was spontaneously depolarized by low external $K^+$, and the magnitude of depolarization was not affected by verapamil, TEA, and a partial replacement of external Na$^+$ and Ca$^{2+}$ with choline chloride. But the membrane potential was hyperpolarized only when the all external cations were substituted with sucrose, and this hyperpolarization was disappeared again by substitution of sucrose with choline chloride. From these results, it may be concluded that the depolarization induced by low external $K^+$ in canine Purkinje fibers is due to the nonspecific increase of membrane permeability to external cations and/or combinations with decreased $K^+$ conductance.

Key Words: Canine Purkinges fiber, low external $K^+$, membrane depolarization.

The membrane permeability to $K^+$ at resting state of excitable membrane is greater than those to any other ions and for this reason the maintenance of resting membrane potential is largely dependent on $K^+$ concentration (Hodgkin and Horowicz 1959). So increase in external $K^+$ concentration depolarize the resting membrane potential and the magnitude of this change bears an almost linear relation to the changes of log $K^+$ concentration and can be predicted from Nernst equation in a certain range of the $K^+$ concentration (Burgen and Terroux 1953a; 1953b). At the same time, it might be expected that a reduction of the extracellular K would hyperpolarize the resting membrane potential, and this has been observed in guinea pig papillary muscle, bullfrog atrial muscle, etc., (Ehara 1974; Eisner and Lederer 1979a; 1979b).

However, in some mammalian heart muscle a reduction of external K induce a depolarization of the membrane potential and generation of spontaneous activity (Vassalle 1965). But the mechanism of this low $K^+$ induced depolarization of resting membrane potential is still uncertain.

There are a few speculations on the possible causes of the depolarization of the membrane potential produced by low external $K^+$, i.e., 1) relative value of $P^*_k$ compared to $P^*_m$ falls at low external $K^+$ (Vaughan-Williams 1959), in which case inward background sodium current may be sufficient to depolarize the membrane potential (Carmeliet 1961; Noble 1979; Fleckenstein 1983); 2) an observed increase in slow inward current in $K^+$-depleted solution may be the cause of depolarization of membrane potential (Goto 1977; Tsuda 1979); 3) the activity of Na-K pump is reduced in low $K^+$ (Barry et al. 1982) and therefore sodium and calcium ions are accumulated in the cell, leading to the generation of spontaneous activity and depolarization of membrane potential. However none of those hypotheses has

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been proved clearly so far.

In this study, the effect of ionic substitution of bathing medium on the membrane depolarization by low external K+ concentration was studied to throw some light on the mechanism of the low K+ induced depolarization.

**METHODS**

1. Solutions

Tris-buffered normal Tyrode solution (pH 7.4) was used in this experiment unless otherwise stated. The compositions of Tyrode solution for the ionic substitution experiment were shown in table 1. In some experiment verapamil and tetraethyl ammonium (TEA) were added in those Tyrode solutions depending on the experimental purposes which will be described in Results section.

<table>
<thead>
<tr>
<th>Table 1. Composition of solutions (in mM)</th>
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<tr>
<td>NaCl</td>
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<td>KCl</td>
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<td>Choline Cl</td>
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<td>CaCl₂</td>
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<td>MgSO₄</td>
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<td>Tris</td>
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<tr>
<td>NaH₂PO₄</td>
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<td>Glucose</td>
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pH of all solutions: 7.4

* All solutions have either 4 mM K+ or zero K+. Each solution with 4 mM K+ was applied first to wash out ECF. Then zero K+ effect was determined as described in the text.

2. Tissue preparations

Guinea pig was sacrificed by a blow on head or dog was anesthetized by intravenous injection of sodium pentobarbital (30 mg/kg), and their hearts were taken out and transferred immediately to normal Tyrode solution oxygenated with 100% O₂. The free running Purkinje fibers from both ventricles of dog heart were dissected and immersed in oxygenated cold (4°C) Tyrode solution until use. The left atrium, right ventricular papillary and trabecular muscle of guinea pig were also used.

3. Measurements of membrane potential

The membrane potential was measured by impaling the cardiac cells with conventional microelectrode made from borosilicate glass tubings (WPI) that had resistances of 10-20 MΩ when filled with 3 M KCl, and the potential was recorded on the Gould chart recorder. The preparations were initially perfused with normal Tyrode solution (4 ml/min) at least for 1 hour to recover from the possible injury during preparation (Fig. 1), and then the effect of low K and ionic substitution on the membrane potential change were measured. The temperature of experiments was maintained at 35°C with temperature-regulated water bath.

**RESULTS**

1. Effect of zero K+ on the resting membrane potential in various cardiac tissue

Fig. 2 shows the simultaneous recordings of membrane potential of guinea pig heart muscle before, during and after perfusion with zero K+ Tyrode solution. When the guinea pig right ventricular papillary muscle was perfused with K+-depleted solution, the resting potential was hyperpolarized from -90 mV to -105 mV within 30 minutes and hyperpolarization was maintained for 30 minutes (Fig. 2-A). The similar hyperpolarizations by zero K+ were observed in left atrial, right ventricular trabecular trabecular muscle of guinea pig (Fig. 2-B,C). However, in dog purkinje fiber when it was perfused with K+-depleted solutions the resting membrane potential was depolarized (~84 mV
Fig. 2. Effects of low external K⁺ on the resting membrane potential of various cardiac tissues. A, guinea pig papillary muscle. B, guinea pig ventricular trabeculae. C, guinea pig left atrium. These tissues show hyperpolarization in resting membrane potential when K⁺ is removed from the perfusate. Vertical bar represents 20 mV. Horizontal bar represents 4 minutes.

2. Effect of TEA and verapamil on the low K⁺ induced depolarization

It has been suggested that an increase in slow inward current (Goto 1977) and a reduction in K⁺ conductance by low external K⁺ (Fleckenstein 1983) may be responsible, at least in part, for the depolarization in K-deficient solution. Therefore, some agents which have known to block those ionic channels were tested in this experiment to see if they had any effect on the low K induced depolarization.

Fig. 3-B shows the effect of K channel blocker, TEA (5 mM) on the membrane depolarization in K-depleted solution (Hille 1984). The magnitude of depolarization was not affected by TEA, but the spontaneous activity during depolarization was disappeared. The same result was obtained with a calcium channel blocker, verapamil (Fig. 3-C).

3. Effects of external cation replacement on the low K⁺ induced depolarization

1) Effects of removal of external Na⁺: Dog Purkinje fiber was initially perfused with modified Tyrode solution in which all Na⁺ were replaced with choline (Sol. A Table 1) to wash out Na⁺ trapped in extracellular space, and then the perfusate was switched to the same zero Na⁺ but K⁺-depleted solution (Sol.

Fig. 3. Effects of verapamil and TEA on the depolarization of dog Purkinje fiber induced by low external K⁺. A, when K⁺ was removed from the perfusate spontaneous action potential was elicited and resting membrane potential was depolarized. B, initial spontaneous action potential was followed by the depolarization of resting membrane potential without spontaneous activities when TEA (5 mM) was added into the zero-K⁺ solution. C, the same observation as in B with verapamil (5 mg/l). Vertical bar represents 100 mV. Horizontal bar represents 4 minutes and 1 second in expanded portions for action potential.
A. Control

B. Zero sodium

C. Zero sodium+zero calcium

D. 100% choline chloride

E. 50% choline chloride +50% sucrose

F. 100% sucrose

Fig. 4. Effects of external cations on the depolarization induced by low external K*.

A, when K* was removed from the perfusate, an initial hyperpolarization of resting membrane potential was followed by depolarization and accompanying spontaneous activities. B, Na* was removed from the perfusate. C, Na* and Ca* were removed from the perfusate. Removal of Na* and Ca* did not cause significant changes in the magnitude of depolarization. D, all cations in the perfusate were replaced by choline chloride. E, all cations were replaced by choline chloride (50%) and sucrose (50%). F, all cations were replaced by sucrose. Vertical bar represents 100 mV. Horizontal bar represents 4 minutes.

A with Zero K*). Removal of sodium in the perfusate caused no significant effect on the magnitude of the low K induced depolarization (Fig. 4-A, 4-B). But in contrast to control (Fig. 4-A), a profound initial hyperpolarization for 1 minutes was seen when perfused with zero Na* and zero K* solution (Fig. 4-B).

Removal of Na* and Ca* (Sol. B) caused qualitatively the same effect on the membrane potential change as Na* removal alone, but the magnitude of initial hyperpolarization was even larger and lasted longer (Fig 4-C).

2) Effects of removal of all cations on the low K* induced depolarization: Fig. 4-D shows the effect of all external cation replacement by choline on the depolarization induced by K* deprivation. When the dog Purkinje fiber was perfused with sucrose and zero K* solution (solution D, see Table 1), the membrane
potential was hyperpolarized rather than depolarized by K⁺-deprivation and lasted for at least 1 minutes (Fig. 4-F), and then the membrane potential was gradually recovered (depolarized) but the membrane potential was never depolarized beyond the membrane potential before the perfusion with the solution D with zero K⁺. This membrane potential change lasted for about 5 minutes and then the membrane potential underwent an oscillatory change (Fig. 4-F).

In summary, the magnitude of hyperpolarization induced by K⁺-depleted and sucrose solution was diminished by substitution of sucrose with choline chloride. The 50% substitution of sucrose with choline chloride in K⁺-depleted solution caused a greater depolarization of the membrane potential than before the substitution (Fig. 4-E), and 100% substitution of sucrose with choline chloride (Sol. C) caused even greater depolarization by K⁺-deprivation (Fig 4-D).

**DISCUSSION**

It has been well known that K⁺ is important to maintain the normal cardiac function (Fisch et al. 1966) and the correlation of K⁺ and cardiac function has been investigated by many cardiac electrophysiologists (Hoffman Suckling 1956; Surawicz et al. 1959; Hoffman 1960; Vassalle 1965). On the contrary to the expectation, a reduction of external K⁺ induces a depolarization and spontaneous activity in some mammalian heart muscle such as dog Purkinje fibers. Therefore the effect of ionic substitution on the low K⁺ induced depolarization was studied here to throw some light on the mechanism of this depolarization.

The membrane potentials of other cardiac tissues, such as guinea pig papillary muscle, was hyperpolarized when perfused with K⁺-deficient solution (Fig. 2). In this tissue, the depolarization and spontaneous activity were developed only when the preparation had been electrically stimulated in K⁺-deficient solution over 60 min (Fig. 5; Fleckenstein 1983).

It has been well known that the low K⁺ reduce the activity of Na⁺-K⁺ pump (Ellis 1977; Glynn 1957). Inhibition of this Na⁺-K⁺ pump results in elevation of internal Na⁺ concentration, promoting the intracellular Ca²⁺ loading via Na-Ca exchange process. This Ca²⁺ overload may lead to transient or oscillatory release of Ca²⁺ from intracellular stores (Kass et al. 1978) and consequently induce transient inward currents and aftercontractions. Further stimulation eventually leads to the depolarization and spontaneous activity of papillary muscle cells (Fleckenstein 1983). So the depolarization of those guinea pig papillary muscle cells may be the result of the Na⁺-K⁺ pump inhibition by low K⁺. However, in dog Purkinje fibers a depolarization of the membrane potential without electrical stimulation was observed when perfused with K⁺-deficient solution (Fig. 3-A; Vassalle 1965).

It has been reported previously that the relative value of Pₑ to PₑNa is decreased at low external K⁺ concentration (Vaughan-Williams 1959) and the membrane resistance was roughly doubled when external K⁺ concentration was reduced from 5.4 mM to 2.7 mM (Vassalle 1965). Furthermore, A decrease in con-
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The conductance of inward rectifier K⁺ channel at low external K⁺ was also reported (McAllister and Noble 1966). These mechanisms may lead to a depolarization of the resting membrane potential. This speculation was supported by the fact that the sodium ions are required for the depolarization at low external K (Carmeliet 1961). However, the external K⁺ of these experiments are still over 1 mM. In this study K⁺ was reduced to lower level, even to zero, and the effects of external cations on the low K⁺ induced depolarization in dog Purkinje fiber was re-investigated in this study. When dog Purkinje fiber was perfused with zero K⁺, zero Na⁺ and zero Ca²⁺ solution (choline and Mg²⁺ were present), initial large hyperpolarization was developed which was not found in control and this hyperpolarization was followed by depolarization (Fig. 4-C). And this initial hyperpolarization was more prominent in Na⁺ and Ca²⁺ removal than Na⁺ alone (Fig. 4-B, C). So it may be due to the negative shift of Eₓ without contribution of background influx of cations to membrane potential by external cation removal. As for the depolarization which followed initial hyperpolarization, the magnitude of depolarization was not significantly different from the value with normal Na⁺ concentration. So it is possible that other external cations, including Ca²⁺ (Fig. 4-B), may also enter the cell to depolarize the membrane. But perfusion with zero K⁺, zero Na⁺ and zero Ca²⁺ solution (Mg²⁺ and choline were present), also leads to depolarization of membrane potential as well as control (Fig. 4-C). In this case choline may be responsible for this depolarization, since a significant choline permeability has been found in cardiac muscle, so choline may also enter the cell as depolarizing charge carrier at zero K⁺ (Bosteels et al. 1970). It is therefore possible that low external K⁺ cause the negative shift of Eₓ and concomitantly increase the membrane permeability to external cations, nonspecifically.

To investigate this possibility, all cations of per fusate were replaced with sucrose (Fig. 4-F), the membrane potential was hyperpolarized by K⁺ deprivation and lasted for at least 1 minutes and then gradually returned to the membrane potential before the K⁺ deprivation, and this hyperpolarizing effect was diminished by the substitution of sucrose with choline chloride (Fig. 4-D, E).

From these result, it may be concluded that the depolarization of membrane potential of dog Purkinje fibers induced by low external K⁺ is due to an increase in nonspecific membrane permeability to external cations and/or combinations with decreased K⁺ conductance.

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