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

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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 substitued 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_{Na} 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, leadng 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 1. Composition of solutions (in mM)

	Tyrode	Sol. A	Sol. B	Sol. C	Sol. D
NaCl	137	–	137	–	–
KCl	*	*	*	*	*
Choline Cl	–	137	–	137	–
$CaCl_2$ $2H_2O$	2.5	2.5	–	–	–
$MgSO_4$ $7H_2O$	1.2	1.2	1.2	–	–
Tris	10	10	10	10	10
NaH_4 PO_4	0.3	0.3	0.3	–	–
Glucose	10	10	10	10	10

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% O_2. 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 ventricullar papillary and trabecullar 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

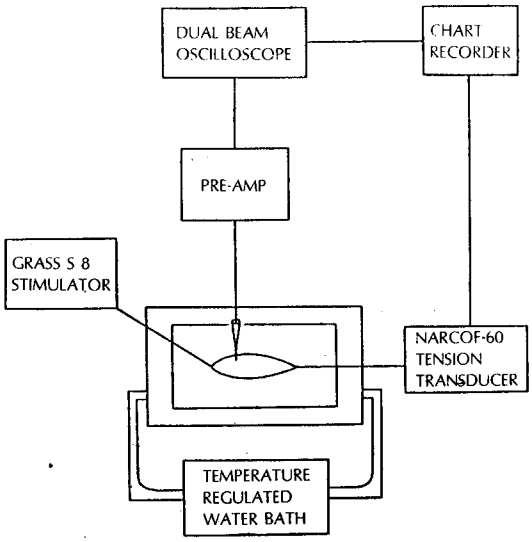

Fig. 1. Schematic diagram of experimental setup for measurements of tensions and membrane potentials.

made from borosilicate glass tubings (WPI) that had resistances of 10-20 M ohm 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 Ko^+ on the resting membrane potential in various cardiac tissue

Fig. 2 shows the simultaneous recordings of membrane potential of gunea pig heart muscle before, during and after perfusion with zero K^+ Tyrode solution. When the guinea pig right ventricullar 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 trabecullar trabecullar 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

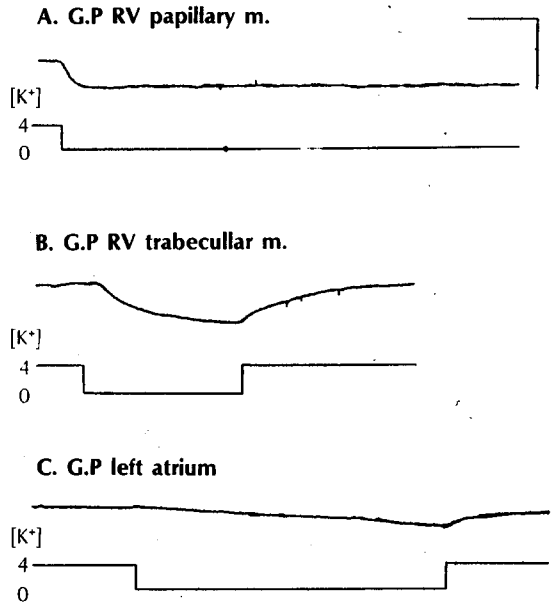

Fig. 2. *Effects of low external K^+ on the resting membrane potential of various cardiac tissues.*
A, guinea pig papillary muscle. B, guinea pig ventricular trabecullae. C, guinea pig left atrium. These tissues show hyperpolarization in resting membrane potential when K^+ is removed from the perfusate. Vertical bar represents 20 mV. Horizontal bar represents 4 minutes.

to −55 mV) and spontaneous activity also appeard (Fig. 3-A).

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.

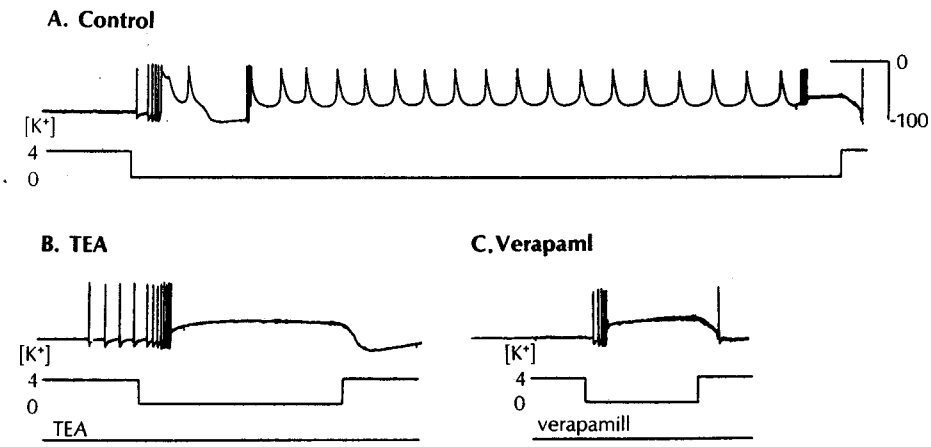

Fig. 3. *Effects of verapamil and TEA on the depolarization of dog Purkinje fiber inducd by low external K^+.*
A, when K^+ was removed from the perfusate spontaneous action potential was elicited and resting membrane potential was depolarized. B, initial spontaneous action potential was followed by the 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.

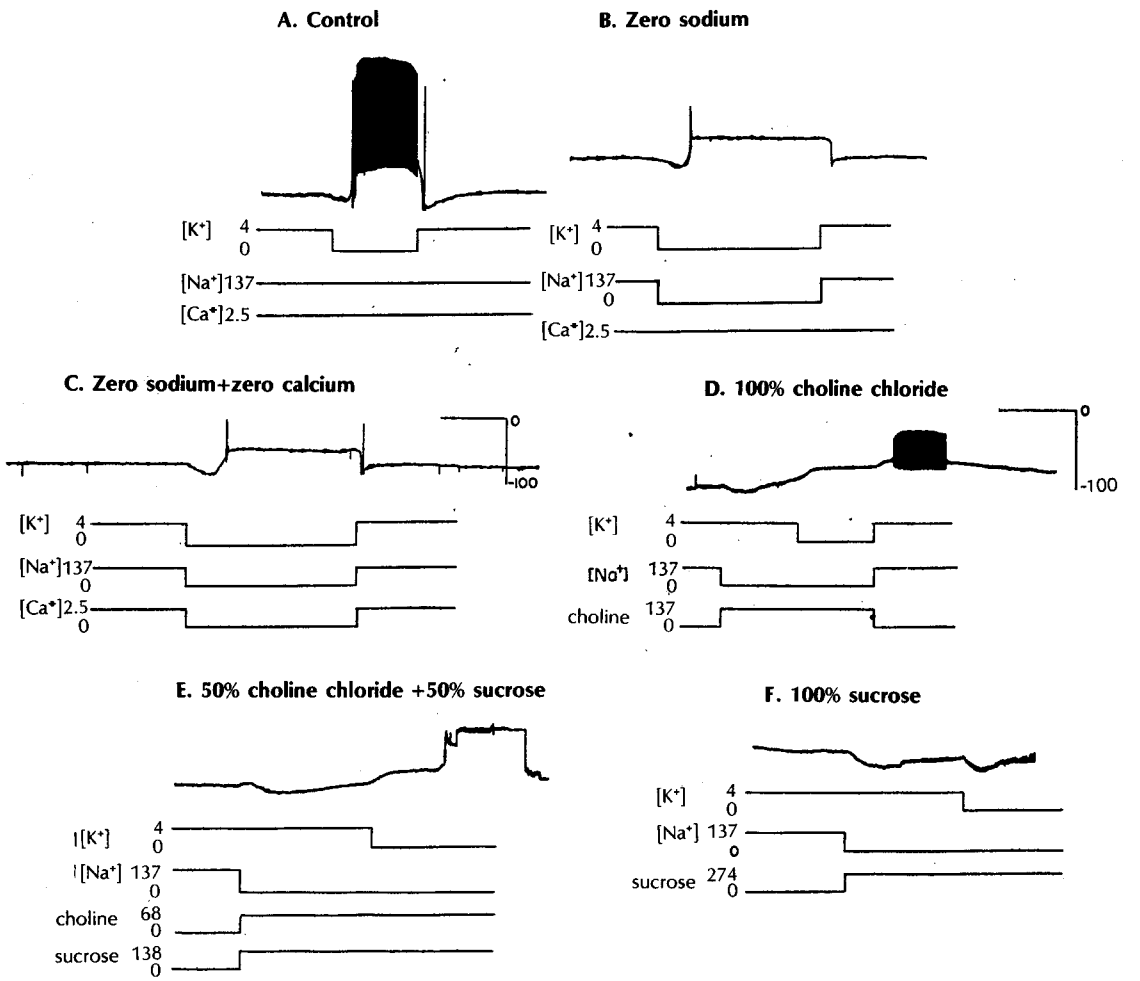

Fig. 4. *Effects of external cations on the depolarization induced by low external K⁺.*
A, when K⁺ was removed from the perfusate, an initial hyperpolarization of resting membrane potential was followed by depolarization and accompanying spontaneous activities. B, Na⁺ was removed from the perfusate. C, Na⁺ and Ca²⁺ were removed from the perfusate. Removal of Na⁺ and Ca²⁺ 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 replace 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 funtion 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 Ca^{2+} loading via Na-Ca exchange process. This Ca^{2+} overload may lead to transient or oscillatory release of Ca^{2+} 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

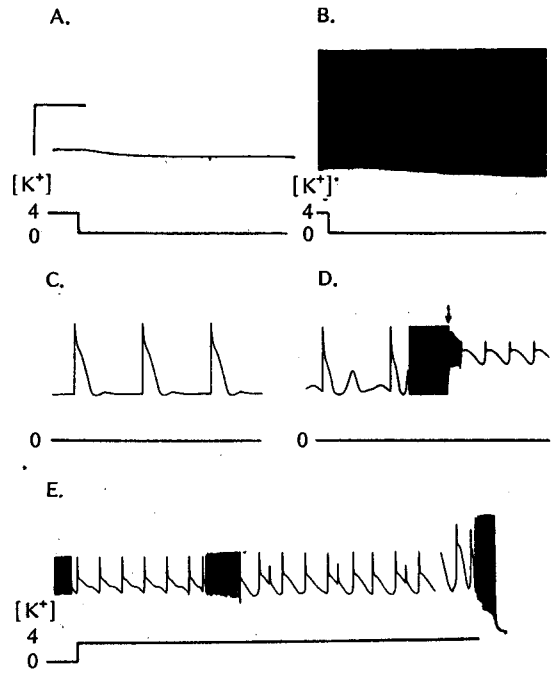

Fig. 5. *Effects of low external K^+ on the membrane potential of guinea pig papillary muscle.*
A, resting membrane potential was hyperpolarized after the removal of K^+ from the perfusate (from −85 mV to −100 mV). B–E, the tissue was electrically stimulated at a frequency of 1 contraction per second. B, resting membrane potential was gradually hyperpolarized in the zero-K^+ solution. C, membrane potentials started to oscillate following action potential (45 minutes after K^+ was removed from the perfusate). D, membrane potential was depolarized with much smaller action potentials (60 minutes after B). E, membrane potential was gradually repolarized after K^+ was added into the perfusate and returned to the normal resting membrane potential. Horizontal scale represents 4 minutes and 1 second in expanded portions. Vertical scale represents 100 mV in A and 20 mV in B–E.

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 P_K to P_{Na} 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-

ductance 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 reivestigated in this study. When dog Purkinje fiber was perfused with zero K^+, zero Na^+ and zero Ca^{2+} solution (choline and Mg^{2+} 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 Ca^{2+} removal than Na^+ alone (Fig. 4-B, C). So it may be due to the negative shift of E_K 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 Ca^{2+} (Fig. 4-B), may also enter the cell to depolarize the membrane. But perfusion with zero K^+, zero Na^+ and zero Ca^{2+} solution (Mg^{2+} 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 E_K and concomitantly increase the membrane permeability to external cations, nonspecifically.

To investigate this possibility, all cations of perfusate 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 substitutition 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 permembility to external cations and/or combinations with decreased K^+ conductance.

REFERENCES

Barry WH, Liechty L, Beaudoin D, Smith TW: Comparison of a low extracellular potassium concentration and cardiac glycoside on contractility, monovalent cation transport, and Na-Ca exchange in cultured ventricular cells. *Trans Assoc Am Physicians 95: 12-21, 1982*

Bosteels S, Vleugels A, Carmeliet E: Choline permeability in cardiac muscle cells of the cat. *J Gen Physiol 55:602-619, 1970*

Burgen ASV, Terroux KG: The membrane resting and action potentials of the cat auricle. *J Physiol 119:139-152, 1953a*

Burgen ASV, Terroux KG: On the negative inotropic effect in the cat's auricle. *J Physiol 120:449-464, 1953b*

Carmeliet EE: Chloride ions and the membrane potential of Purkinje fibers. *J Physiol 156:375-388, 1961*

Ehara T: Late potentiating effects of low-K Ringer on the contractility of the bullfrog ventricle. *Jap J Physiol 294:255-277, 1974*

Eisner DA, Lederer WJ: Inotropic and arrhythmogenic effects of potassium depleted solutions on mammalian cardiac muscle. *J Physiol 294:255-277, 1979a*

Eisner DW, Lederer WJ: The role of the sodium pump in the effects of potassium depleted solutions on mammalian cardiac muscle. *J Physiol 294:279-301, 1979b*

Ellis D: The effects of external cations and ouabain on the intracellular sodium activity of sheep heart Purkinje fibers. *J Physiol 273:211-240, 1977*

Fisch C, Knoebel SB, Feigenbaum H, Greenspan K: Potassium and the monophasic action potential, electrocardiogram, conduction and arrhythmia. *Prog Cardiovas Dis 8:387-418, 1966*

Fleckenstein A: *Calcium antagonism in heart and smooth muscle.* John Wiley & Sons, Inc. *1983*

Glynn IM: The action of cardiac glycosides on sodium and potassium movements in human red cells. *J Physiol 136:148-173, 1957*

Goto M, Tsuda Y, Yatani A: Two mechanisms for positive inotropism of low-K Ringer in bullfrog atrium. *Nature 268:755-757, 1977*

Hille B: *Ionic channels of excitable membranes.* Sinauer Associates Inc. *1984.*

Hodgkin AL, Horowicz P: The influence of potassium and chloride ions on the membrane potentials of single muscle fibers. *J Physiol 148:127-160, 1959*

Hoffman BF: *Electrophysiology of the heart.* McGrow-Hill Book Company, Inc. *1960.*

Hoffman BF, Suckling EE: Effects of several cations on transmembrane potentials of cardiac muscle. *Am J Physiol 186:317-324, 1956*

Kass RS, Lederer WJ, Tsien RW, Wiengart R: Role of calcium ions in transient inward currents and afftercontractions

induced by strophanthidin in cardiac Purkinje fibers. *J Physiol 281:187-208, 1978*

McAllister RE, Noble D: The time and voltage dependence of the slow outward current in cardiac purkinje fibers. *J Physiol 186:632-662, 1966*

Noble D: *The initiation of the heart beat.* 2nd edition. Clarendon Press, Oxford. *1979*

Surawicz B, Lepeschkin E, Herrlich HC, Hoffman BF: Effect of potassium and calcium deficiency on the monophasic action potential, electrocardiogram and contractility of isolated rabbit hearts. *Am J Physiol 196:1302-1307, 1959*

Tsuda Y: The nature of the initial positive inotropic effects of K depletion in Bullfrog atrial muscle. *Jap J Physiol 29:103-117, 1979*

Vassalle M: Cardiac pacemarker potentials at different extra- and intracellular K concentrations. *Am J Physiol 208:770-775, 1965*

Vaughan-Williams EM: The effects of changes in extracellular potassium concentration on the intracellular potentials of isolated rabbit atria. *J Physiol 146:411-427, 1959*