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PARADOXICAL INOTROPIC RESPONSE OF HEART TO K^+

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PARADOXICAL INOTROPIC RESPONSE OF RAT HEART TO K+

By

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ABSTRACT

PARADOXICAL INOTROPIC RESPONSE OF RAT HEART TO K+

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In isolated heart muscle preparations obtained from various species, raising the extracellular K^+ concentration ($[K^+]_o$) causes a negative inotropic effect. It is proposed that the negative inotropic effect of K^+ is due to a stimulation of the Na pump. In the left atrial muscle of rat heart, however, raising $[K^+]_o$ from 3.5 mM to 9.5 mM caused a positive inotropic effect (PIE). Therefore, the mechanism(s) of this unique response of rat heart to K^+ was studied to re-evaluate the above hypothesis. At lower concentrations (from 1 mM to 3 mM), raising $[K^+]_o$ caused a negative inotropic effect consistant with the above hypothesis. However, further increase in $[K^+]_o$ caused a PIE. Riaising $[K^+]_o$ (3.5 mM to 9.5 mM) caused a 20 mV depolarization of the resting membrane potential and a prolongation of the action potential duration (APD) in rat heart, in contrast to shortening of APD observed in most species. Voltage clamp experiments revealed a unique early transient outward current (I_{EO}) in the isolated rat myocytes. Inactivation of this outward current by the membrane depolarizing effect of K^+ seems to be responsible for the APD lengthening effect of K^+. When the I_{EO} was eliminated by 4-aminopyridine (3 mM) K^+ still produced a PIE suggesting that the elimination of I_{EO} is not a major factor in the PIE of K^+. Conditions that increase the relative Na load of the Na pump such as

increasing the stimulation frequency, decreasing the incubation temperature or preincubation with a Na channel openor (veratridine), attenuated or abolished the PIE of K^+. Under the condition in which the intracellular Na^+ load was minimized by low frequency of stimulation, K^+ produced a PIE in the guinea-pig left atrial muscle preparations. When the atrial preparations of rat were preincubated with Cd^{2+} (20 μM), K^+ produced only a transient PIE. Caffeine (3 mM) but not ryanodine (6 nM) abolished the K^+-induced PIE. It is concluded that the negative PIE of K^+ is masked by a strong positive PIE in the rat heart, that $[Na]_i$ plays a modifying role in the PIE of K^+, and that certain specific pools of Ca^{2+} seem to be important in the PIE of K^+.

to my lovely wife, Olivia

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Mammalian cardiac cells, like other animal cells, have an intracellular Na^+ concentration ($[Na^+]_i$) that is substantially lower than that in the extracellular fluid ($[Na^+]_o$). The intracellular K^+ concentration ($[K^+]_i$), in contrast, is higher than the extracellular K^+ concentration ($[K^+]_o$). Therefore, there are steep ion gradients of Na^+ and K^+ directed in two opposite directions. This gradient is partly maintained by the relative impermeability of the sarcolemmal membrane to these two ions. However, the active counter-transport of Na^+ and K^+ by Na,K-ATPase or the "sodium" pump is the major factor in maintaining the gradient (Glynn and Karlish, 1975; Wallick et al., 1979; Akera and Brody, 1982; Glitsch, 1982). Such a transport mechanism is especially important in excitable cells such as cardiac muscle cells because significant Na^+ gain and K^+ loss occur during the excitation-contraction process as the result of passive ion fluxes.

A. Regulation of the Na Pump

The most important regulators of Na pump activity are Na^+ and K^+ions (Glitsch, 1979; Wallick et al., 1979; Akera and Brody, 1982). Although the concentrations or availability of other substrates may also influence Na pump activity, the importance of their regulatory role is questionable. For instance, ATP is the energy source for the transport process and also is known to affect the affinity of Na^+ and K^+ binding to the enzyme (Robinson, 1977; Skou, 1979; Hastings and Skou, 1980). However, the cytoplasmic ATP concentration is about five times higher

than the K_d value for the low affinity binding sites for ATP (Skou, 1979). Mg^{2+}, inorganic phosphate and Ca^{2+} also can affect the Na,K-ATPase activity (Godfraind et al., 1977; Robinson and Flashner, 1978; Flashner and Robinson, 1979; Robinson and Flashner, 1979); however, the intracellular concentrations of these ions are unlikely to change to such an extent that the Na pump activity would be significantly affected.

Since the Na pump regulates the intracellular Na^+ concentration, it is not surprising that $[Na^+]_i$ sets the activity of the Na pump. In squid giant axons, it has been shown that an increase in $[Na^+]_i$ stimulates active Na pump transport (Brinley and Mullins, 1968; Baker et al., 1969). Varying the $[Na^+]_i$ by cooling the guinea-pig atrial tissue in buffer containing different $[Na^+]_o$, Glitsch et al. (1976) demonstrated that Na pump activity, measured by Na^+ efflux, was a function of the $[Na^+]_i$. The half maximum activation of the pump was about 22 mM which is much higher than the K_m value (0.16-8.1 mM) estimated in isolated enzyme preparations (Robinson and Flashner, 1978). Because recent intracellular ion selective electrode data indicate that $[Na^+]_i$ is in the range of 8-10 mM (Cohen et al., 1982; Eisner, et al., 1984; January and Fozzard, 1984), a change in $[Na^+]_i$ is thus likely to change pump activity. Interventions which increase Na^+ influx, such as increasing stimulation frequency or using a Na^+ or cation ionophore, augment Na pump activity (Yamamoto et al., 1979; Akera et al., 1981).

There is also no doubt that $[K^+]_o$ also controls the activity of the Na pump. It has been shown repeatedly that depletion of $[K^+]_o$ leads to inhibition of the Na pump and an elevation of $[K^+]_o$ leads to stimulation (Deitmer and Ellis, 1978; Eisner and Lederer, 1979a; Eisner and Lederer,

1979b; Eisner and Lederer, 1980; Gadsby, 1980; Glitsch, et al., 1981; Daut, 1983; Eisner et al., 1984). However, a controversy still exists as to the precise $[K^+]_o$ that half-maximally activates the pump. This in part results from the fact that there are basically two different methods for studying the activation of the pump. In one method, the current underlying the transient hyperpolarization resulting from the electrogenic Na pump activity is analyzed by voltage clamp techniques (Eisner and Lederer, 1979b; 1980b; Gadsby and Cranfield, 1979a, b; Glitsch, et al., 1978). Alternatively, the changes in $[Na^+]_i$ activity during the reactivation of the pump can be measured using intracellular Na^+ ion sensitive electrodes (Ellis, 1977; Deitmer and Ellis, 1978; Glitsch, et al., 1981). From the changes in intracellular Na^+ ion activity, Deitmer and Ellis (1978) concluded that the half-maximum activation of the Na pump by K^+ in the sheep Purkinje fibers was 10-12.5 mM. However, using the same technique, Glitsch et al. (1981) showed that half-maximum activation occurred at 1.6-3.7 mM K^+ or Rb^+. Gadsby (1980b), analyzing the Na pump current, concluded that $[K^+]_o$ at 1 mM half-maximally activates the Na pump. Using the same technique, however, Eisner and Lederer (1980b) obtained a K_m value of 6 mM Rb^+. Because $Rb^+$and K^+ seem to activate the pump at a similar potency, 6 mM Rb^+ probably is equivalent to 6 mM K^+. Most of their K_m values were apparently higher than obtained from isolated enzyme studies which are in a range of 1 mM K^+.

In most species, the normal plasma $[K^+]$ is at the range of 4-6 mM. Variation of plasma K^+ may or may not change the activity of the Na pump depending on the effective K_m values. If the K_m for K^+ activation of the Na pump is actually around 1 mM, then an increase in plasma $[K^+]$ is

unlikely to further stimulate the Na pump. However, if the half-maximum activation of the Na pump is in the 6-10 mM range, a change in $[K^+]_o$ is likely to stimulate the Na pump. The well-known case of $[K^+]_o$ affecting the Na pump is demonstrated in skeletal muscle. During exercise, K^+ in the plasma can rise to about 10 mM. Under these conditions, the Na pump in the skeletal muscle cell is stimulated by $[K^+]_o$ which is transported quickly into the cell (Kjeldsen et al., 1984). In cardiac tissue, the physiological regulation of the Na pump by $[K^+]_o$ has not been clearly demonstrated.

B. The Na Pump and Cardiac Contractility

How Na pump activity affects the contractility of the heart has been the subject of a large number of recent publications (Deitmer and Ellis, 1978; Eisner and Lederer, 1979b; Eisner and Lederer, 1980b; Eisner et al., 1981; Cohen et al., 1982; Lee and Degostino, 1982; Wasserstrom, et al., 1983, Eisner et al., 1984). A good correlation has been established between $[Na^+]_i$ and contractility. One of the most useful tools in this field of study has been the cardiac glycosides. Although some controversies still exist regarding the exact mechanism(s) of the positive inotropic action of the cardiac glycosides (Okita, 1977; Noble, 1980; Hart etal., 1983), it is now widely accepted that inhibition of the Na pump probably plays the most important role (Akera, 1977; 1981). Inhibition of the Na pump is thought to increase the $[Na^+]_i$ and, subsequently, the $[Ca^{2+}]_i$. This is the so-called "Na pump lag" hypothesis which was first advanced by Langer (1965; 1968; 1983). The recent work cited above tends to support such a mechanism. Using intracellular Na^+ ion selective electrodes, an increase in $[Na^+]_i$ has

been correlated with a simultaneous increase in contractile force in Purkinje fibers (Eisner et al., 1981; Cohen et al., 1982).

Strong evidence suggests that the Na^+-Ca^{2+} exchanger is the link between the elevation of $[Na^+]_i$ and the $[Ca^{2+}]_i$ increase. Data from isolated sarcolemmal vesicles (Reeves and Sutko, 1979) and intact cardiac tissue (see Langer, 1982) indicate the existence of a Na^+-Ca^{2+} exchanger. This exchanger is able to move ions across the membrane in either direction depending on the electrochemical gradient. Ca^{2+} moves into the cell via the Na^+-Ca^{2+} exchanger during the systolic phase and out of the cell during the diastolic phase. Elevated $[Na^+]_i$, such as that caused by Na pump inhibition by cardiac glycosides or $[K^+]_o$ depletion, would increase Ca^{2+} influx and reduce Ca^{2+} efflux. The end result is an increase in $[Ca^{2+}]_i$ (Langer, 1982).

C. Paradoxical Response of Rat Heart to $[K^+]_o$

While studying the effects of K^+ on inotropic effects of different cardiac glycosides, we observed an increase in developed tension of rat atrial muscle preparations when $[K^+]_o$ was increased (see Akera et al., 1979, Figure 7). In the above study, the average developed tension of atrial muscle preparations incubated in a medium containing 3.5 mM K^+ was 0.94 ± 0.05 g whereas that observed in 9.5 mM K^+ was 1.23 ± 0.10 g. It should be noted that these values were obtained with separate groups of atrial muscle preparations; however, when experiments were performed specifically to examine the effect of K^+ on the developed tension, the results confirmed the above findings.

These results were rather unexpected. As has been discussed earlier, an elevation of $[K^+]_o$ would stimulate the Na pump and lead a

negative inotropic effect because $[Na^+]_i$ would be reduced. Depending on the K_m value for the K^+-induced activation of the Na pump, a change in $[K^+]_o$ from 3.5 mM to 9.5 mM may or may not affect the Na pump and, subsequently, the developed tension may or may not be decreased. However, a positive inotropic effect induced by raising $[K^+]_o$ was rather unexpected.

There are scattered reports on the K^+-induced positive inotropic effect (PIE) observed in cardiac tissue, although the nature of the PIE seems to be different. Kavalar et al. (1972) reported a K^+-induced transient PIE in cat papillary muscle when $[K^+]_o$ was raised from 4 mM to 8 mM. In an attempt to explain the observation, they developed a so-called "electrically buffered" papillary muscle preparation. A papillary muscle was separated into a long and short segment by a rubber partitioning diaphragm. When K^+ was applied to the long segment there was a small shortening of action potential duration (APD) and a reduction in action potential amplitude (APM). Associated with these changes, a negative inotropic effect was observed in almost all preparations. When K^+ was added to the short segment, there were no change in either APD or APM due to the "buffering" effect of the long segment. However, a transient PIE could be seen in most of the preparations. The investigators concluded that a change in APD and APM probably play a role in the K^+-induced negative inotropic effect. Interestingly, these investigators did not consider Na pump stimulation as a possible explanation for the negative inotropic effect. The PIE did not seem to correlate with changes in APD or APM and no explanation was offered as a mechanism for the K^+-induced PIE. Ku etal. (1975) also observed a transient increase in developed tension of a left atrial

muscle preparation of guinea pig when $[K^+]_o$ was increased from 5.8 mM to 10.8 mM; it was followed by a sustained negative inotropic effect. The transient PIE was only partially blocked by propranolol pretreatment. Again no explanation was given for the K^+-induced PIE.

Therefore, it would appear that the primary response of mammalian heart muscle to an elevation of K^+ in many species studied is a negative inotropic effect. This is presumably due to stimulation of the Na pump by $[K^+]_o$. Rat heart muscle was the only preparation examined so far to respond with a sustained PIE when $[K^+]_o$ was elevated. This paradoxical response of rat heart to an elevation of $[K^+]_o$ suggests that either stimulation of the Na pump by K^+ in this species is different from that in other species and/or K^+activates a process unique to the rat heart to increase contractile force. It is well known that rat heart muscle has many characteristics different from other species. Therefore, it is tempting to speculate that the paradoxical K^+response is related to these differences in the rat heart. The excitation-contraction coupling mechanism of the rat heart is probably not totally different from that in hearts of other species, but rather the difference may derived from some "altered" function in the excitation-contraction coupling mechanism or in the contractile system. An understanding of these species-dependent differences is therefore likely to facilitate knowledge of the excitation-contraction process in cardiac muscle.

D. The Unique Characteristics of the Rat Heart

The rat myocardium has specific characteristics compared to other species which indicate some differences in biochemistry and electrophysiology involved in membrane excitation, the

excitation-contraction coupling mechanism and in contractile activation. For example, the rat myocardium has a short APD which is only of 50-100 ms duration (Kelly and Hoffman, 1960; Langer et al., 1975a). In addition, the action potential lacks a plateau phase which is prominent, as in other species (Coraboeuf and Vassort, 1968). The plateau phase is generally believed to be maintained by the slow inward current (Reuter, 1973; 1979). Whether the lack of the plateau phase of the action potential in the rat heart is due to a smaller slow inward current compared to other species, or due to the existence of an outward current, is not well established.

The rat heart is also known to exhibit a negative force-frequency relationship, i.e. when stimulation frequency is increased, there is a decrease in developed tension (Blesa et al., 1970; Forester and Mainwood, 1974; Langer et al., 1975a). In myocardium of other species, a positive force-frequency relationship is observed. It is generally believed that the positive staircase seen in these species is the result of the "Na pump lag". Blesa etal. (1970) found no net K^+ loss (indication of no net Na^+ gain) in the rat ventricular myocardium when stimulation frequency was increased. They suggested that the absence of a "Na pump lag" in the rat heart could be an explanation for the unique force-frequency relationship in the rat heart.

The rat heart is about 500 fold less sensitive to digitalis than other species. The low affinity of the cardiac Na,K-ATPase for the glycoside, resulting from a high dissociation rate constant of the glycoside-enzyme complex (Akera, 1977) has been suggested to be the major contributing factor for the low sensitivity of the rat heart to cardiac glycosides (Akera et al., 1979). However, mechanisms unrelated

to the binding site affinity have also been proposed. Langer et al. (1975a) have proposed a common mechanism to explain these unique characteristics of the rat heart, i.e. the short APD, the negative force-frequency relationship and the low sensitivity to the cardiac glycoside. They demonstrated that all "paradoxical" responses disappeared from the neonatal rat heart which seemed to have "normal" electrophysiological properties. The APD of ventricular tissue of neonatal rat heart stimulated at 1.5 Hz was 243 ms at the age of 3.2 days. The APD shortened to 147 ms at the age of 20 days (see Langer, 1975a). Langer reported that a positive force-frequency relationship could be demonstrated between 0.5 to 1.5 Hz in 5 days rats. The neonatal rat heart has a higher sensitivity to ouabain compared to the adult rat heart. All these characteristics of neonatal rat heart could be converted to that of the adult rat heart when $[Na^+]_o$ was reduced. Furthermore, ouabain caused a net ^{42}K loss from the neonatal rat heart which could not be demonstrated in the adult rat heart. These investigators therefore proposed that a slow Na^+ channel which existed in the neonatal rat heart became suppressed with age. The adult rat heart would therefore have less tendency to accumulate Na^+ during either high frequency stimulation or treatment with digitalis. Thus, the absence of "Na pump lag" seems to be capable of explaining the many unique characteristics of the adult rat heart.

On the other hand, evidence has also been presented to suggest a dissociation of the positive staircase from the digitalis (ouabain)-induced PIE. McCans et al. (1974) showed that in the presence of a Ca^{2+} channel blocker, verapamil, the positive staircase of rabbit ventricular muscle preparation reversed to a negative staircase.

Despite the fact that rat heart shows a negative staircase in the presence of verapamil, verapamil did not alter the glycoside sensitivity of the rabbit heart. However, in view of more recent knowledge about the mechanism of action of verapamil, interpretation of this data is probably incorrect. It has been shown by different investigators (Bayer et al., 1975; Ehara and Kaufman, 1978; Linden and Brooker, 1980; Lee and Tsien, 1983) that the effectiveness of several Ca^{2+}channel blockers, including a derivative of verapamil (D600), was enhanced by repetitive depolarization - so-called "use dependence". Therefore, the force of contraction of the rabbit heart muscle preparation is expected to decrease when the frequency of stimulation is increased, because the effectiveness of verapamil is expected to be enhanced. Thus, the observation of McCans et al. cannot be an indication of a dissociation between the positive staircase and the digitalis-induced PIE.

The mechanism of the K^+-induced PIE in the rat heart muscle is unknown. Whether this effect of K^+ is related to the negative force frequency relationship of the rat is not clear. Whether there is any relationship between the paradoxical response of rat heart to K^+ and the lack of "Na pump lag" in the rat heart, assuming the hypothesis to be correct, remains to be explored.

E. Objectives

The objective of the present study was to elucidate the mechanism(s) underlying the K^+-induced PIE observed in the isolated rat heart muscle. In order to achieve the above objective, the following four specific aims were pursued.

1. To characterize the K^+-induced PIE in the rat heart.

2. To determine the relationship between Na pump activation and the K^+-induced PIE.

3. To explore possible mechanism(s) of the PIE of K^+ and its relationship to other unique characteristics of the rat heart.

4. To determine whether any specific pool(s) of Ca^{2+} is involved in the K^+-induced PIE.

It was hoped that by solving these problems a better understanding of the excitation-contraction coupling process in general could be achieved.

MATERIALS and METHODS

A. Studies on the Force of Contraction

Atrial muscle preparations were obtained from male Sprague-Dawley rats weighting 300-350 g or from guinea pigs of either sex weighting 300-350 g. The animals were stunned by cervical dislocation and their hearts immediately removed. Left atrial muscle was excised and suspended vertically in a bath containing Krebs-Henseleit (K-H) solution of the following millimolar composition: NaCl, 118.00; $NaHCO_3$, 27.20; $MgSO_4$, 1.20; KH_2PO_4, 1.00; $CaCl_2$, 1.80 and glucose, 11.10. Concentration of KCl is as indicated in each experiment. The solution was maintained at 30°C or as indicated in specific experiments and aerated with a 95% O_2-5% CO_2 gas mixture which produced a pH of 7.4. The preparations were field-stimulated at the frequency indicated for each experiment with square-wave pulses of 4 ms duration at a voltage 30% above threshold. Nearly isometric force of contraction was recorded with a force-displacement transducer and a polygraph recorder (FT03C and model 7B, respectively, Grass Instruments Co., Quincy, MA). Resting tension was adjusted to 1.0 g during the 45- to 60-min equilibration period.

B. Na,K-ATPase Studies

Na,K-ATPase preparations were obtained from ventricular muscle of rats (300-325 g) or guinea pigs (300-350 g) with deoxycholate and NaI treatment as described by Akera *et al.* (1978). ATPase activity was assayed at 37°C by incubating enzyme preparations (0.1 mg of protein per ml) in a 1.0 ml medium containing 5 mM Tris-ATP, 5 mM $MgCl_2$, 100 mM

NaCl, 50 mM Tris-HCl Buffer (pH 7.5) and various concentrations of KCl as indicated. After a 10 min pre-incubation period in the absence of KCl, the ATPase reaction was started by the addition of KCl to the mixture (Akera et al., 1979). Mg-ATPase activity was assayed in the presence of 3 mM ouabain and in the absence of K^+. The reaction was terminated by the addition of trichloracetic acid (final concentration, 7.5%). The mixture was chilled immediately in an ice-bath and the amount of inorganic phosphate (P_i) released from ATP was assayed by the method of Bonting et al. (1961). Na,K-ATPase activity was calculated as the difference between total and Mg-ATP activity.

C. Isolated Myocyte Preparation

The isolation method is adapted from that described by Powell and Twist (1976). Male Sprague-Dawley rats weighting 300-350 g were killed by cervical dislocation. Hearts were removed quickly and perfused retrogradely through the aorta at 37°C with the above K-H buffer but containing 2.5 mM Ca^{2+}. The perfusion pressure was set at 60 cm H_2O. The hearts were perfused for about 10 mins until all visible blood was cleared. The perfusion was then quickly switched to a Ca^{2+} free K-H buffer for 10 mins (about 100 ml) at a perfusion pressure of 80 cm H_2O. Subsequently, 50 ml of collagenase and 30 mg of hyaluronidase was added to 100 ml of the Ca^{2+} free K-H buffer solution. The preparation was perfused for 40 mins by recirculating the perfusate. The tissue was then cut into small pieces and incubated for an additional 10 mins at 37°C in a modified K-H solution containing 1 mM Ca^{2+}. Incubation was continued for another 15 mins with the heater turned off and maintained at room temperature. The enzyme-containing K-H solution was then

decanted off and a fresh K-H buffer containing 1 mM Ca^{2+} without the enzymes was added. A piece of the tissue was taken out and agitated in the K-H solution to release cells each time when cells were needed. Cells usually appear viable (rod shape with clear striation, quiescent and responded to electrical stimulation) from 3 to 5 hours under this condition. Atrial cells are usually more difficult to obtain. Whereas the yield for viable ventricular cells usually was around 20-50%, the yield for viable atrial cells varied from 5-20%.

D. Electrophysiological studies in multicellular preparations

Left atrial muscle preparations of either guinea-pig or rat heart were obtained as described above. Atrial preparations were horizontally suspended in a chamber containing 3 ml of the K-H buffer with 1.8 mM Ca^{2+} and 3.5 mM K^{+}. A continuous flow of fresh incubation medium was added to the chamber by means of an infusion pump (Miniplus II, Gilson Medical Electronics, Inc., Middleton, WI) allowing excess medium to overflow. The incubation chamber was either at room temperature (26oC) or adjusted to 30oC.

Transmembrane potentials were recorded with a floating glass microelectrode which was filled with 3 M KCl and having a tip resistance of 20-25 mega-ohms. The potential was recorded using a model 707 electrometer amplifier (W.P. Instrument, Inc., New Haven, CT), model 502A oscilloscope (Tektronix, Inc., Beaverton, OR) and a Grass model C4R Kymograph camera. The resting membrane potential and duration of action potentials were analyzed either using a Lab 8/e microcomputer (Digital Equipment Corp., Maynard, MA) or manually from oscillograms.

E. Electrophysiological Studies with Isolated Myocytes

The method was basically the same as that described by Hume and Giles (1983). Microelectrodes with short shanks (3-5 uM) were pulled by using a single-turn heater coil on a vertical pipette puller (David Kopf Instruments, Tujunga, CA). Radnoti Glass Technology, Inc., (Monrovia, CA) microstar capillary tubing was used to make the micro-electrodes so that back-filling of the electrodes with 3 M potassium-gluconate could be facilitated. Microelectrodes possessing resistances in the range of 10 megohms were used. Maximum series resistance error with a current of 1 nA would be in the range of 10 mV. Simultanous voltage traces with the current traces indicate constant voltage clamps have always been achieved, i.e. no inflection on the voltage trace which would indicate loss of voltage control.

F. Experimental Procedure and Set Up

The experimental set up was basically the same as that described by Hume and Giles (1983). Low resistance microelectrodes were mounted in a microelectrode holder while was equipped with a suction port (EH-900R; W-P Instruments, Inc., New Haven, CT). A polyethylene tubing was connected from the suction port of the microelectrode holder to a 1-ml or 5-ml gas tight syringe (Hamilton, 1001; A-M Systems, Toledo, OH), which was used to produce a graded negative pressure. The microelectrode holder was back-filled with 2 M potassium gluconate and connected to a preamplifier (KS-700, W-P Instruments, Inc.), the headstage of which was mounted on the stage of an inverted microscope (Swift Instruments Ltd., San Jose, CA). After applying suction to attach the electrode on the surface of the isolated myocyte, the cell

membrane on the pipette tip ruptured, the voltage signal from the suction micropipette was led to the (-) side of a FET input unity gain amplifier (KS-700, W-P Instruments, Inc.). The membrane potential signal, -Vm, was then compared with the rectangular command voltage level using a differential amplifier (AM502., Tektronix Inc., Beaverton, OR) having a variable gain and bandwidth. Typically, this feedback amplifier was run at a gain of 300 with a bandwidth of DC-3 KHz. The error signal was applied to the suction micropipette via the current-passing section of the amplifier. Rectangular pulses were generated by applying the TTL pulses from a digital clock (W-P Instruments, Inc.) to a voltage divider circuit, which had an optical isolation unit at its output. The holding potential was varied by applying a 5 V DC level to a second voltage divider circuit. Membrane current was measured as the voltage drop across a one megohm resistor positioned at the output of the feedback amplifier (for circuit diagram see Hume and Gile, 1983). Membrane potential and current signals were monitored by using a digital processing oscilloscope (Nicolet 4094, Nicolet Instrument Corp., Madison, WI).

RESULT

A. Positive Inotropic Effect of K^+ on the Rat Heart

To confirm the earlier result that K^+ causes an increase in developed tension in the rat heart, left atrial muscle preparation obtained from the rat heart was equilibrated in a Krebs-Hensleit buffer containing 3.5 mM K^+. As shown in Figure 1, K^+ indeed induced a positive inotropic effect when $[K^+]_o$ was elevated from 3.5 mM to 9.5 mM. In several preparations, a large transient positive inotropic effect preceded the more stable inotropic response. The increase in contractility usually lasted for 25 mins or more. To test whether this positive inotropic effect of K^+ was reversible, a superfusion tissue bath system was used. In addition, the effect of K^+ on the atrial muscle of guinea pig was compared to that of rat. Since a long incubation of guinea-pig atrial muscle in a medium containing less than 4 mM K^+ caused extrasystoles to occur (data not shown), guinea-pig preparations were first equilibrated in a medium containing 9.5 mM K^+. As the K^+ concentration of the medium was decreased to 6 and then to 3 mM by reducing the rate of infusion Of K^+, force of contraction of the preparation was increased as the concentration of K^+ was decreased (Figure 2). When the K^+ concentration was increased stepwise back to 6 and 9 mM after a brief incubation of the guinea-pig atrial muscle preparations in a medium containing 3 mM K^+, developed tension was decreased. K^+ ion had an opposite effect on left atrial muscle preparations obtained from rats. The preparations were equilibrated in a K-H buffer containing 3 mM K^+ (incubation of this

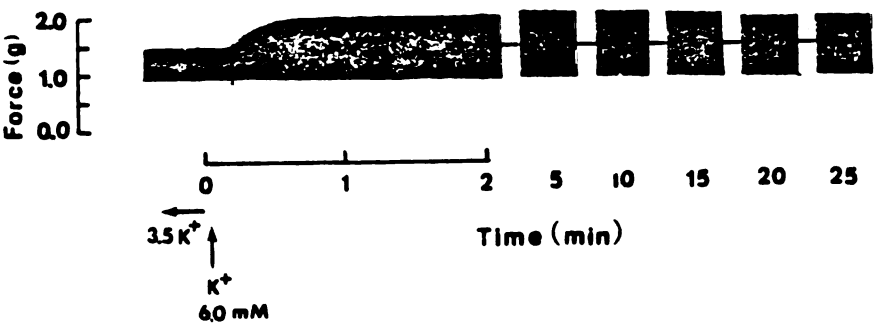

Figure 1

Figure 1. A typical tracing of the effect of K^+ on the developed tension of rat heart. Left atrial muscle preparations of rat were equilibrated at 30°C in a K-H buffer containing 3.5 mM K^+. Atria were electrically stimulated at 1.5 Hz. After a 60 min equilibration period, the K^+concentration was elevated by 6 mM to a final concentration of 9.5 mM at time zero.

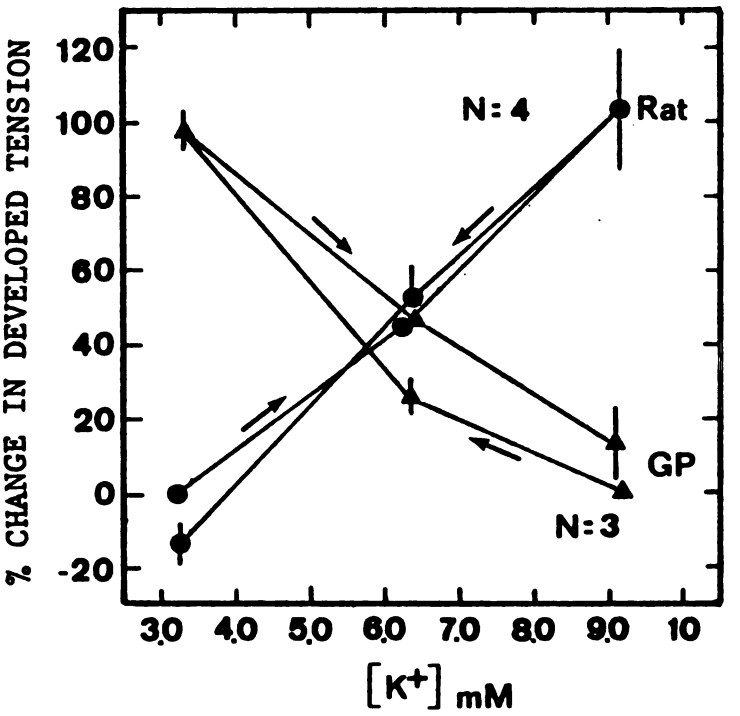

Figure 2

Figure 2. Effects of K^+ superfusion on rat and guinea-pig atrial muscle preparations at 30°C. Atrial muscle preparations of rat were electrically stimulated at 1.5 Hz in a K-H buffer containing 3 mM K^+. The K-H buffer was superfused into the incubation chamber continuously allowing overflow of the buffer. After a 45 min equilibration period, K^+ was infused into the superfusing K-H buffer to increase the $[K^+]$ to 6 and 9 mM by changing the pre-set perfusion rate. Each step change required 8 to 10 mins for the developed tension to reach equilibrium. The $[K^+]$ was then stepped down to 6 and 3 mM by reducing the infusion rate of K^+. Similar protocol was applied to left atrial muscle preparations of guinea-pig, except the atrial preparation was first equilibrated for 45 mins in a K-H buffer containing about 9 mM K^+. The changes in developed tension were expressed as percentages of the contractile force at 3 mM and 9 mM K^+ for rat and guinea-pig atria, respectively. Vertical lines indicate standard error of the mean. Each point represents 3 to 4 experiments.

tissue in K-H buffer with low K^+ did not lead to extrasystoles). As the concentration of K^+ was increased to 6 and then to 9 mM, there was a stepwise increase in the force of contraction. This increase in contractility was readily reversible; as the concentration of K^+ was decreased, there was a corresponding decrease in developed tension. Therefore, an elevation of K^+ concentration in the range between 3 mM and 9 mM decreases the developed tension in guinea pig atrial muscle preparations obtained from guinea-pigs but increases it in similar preparations from rat heart.

There was a possibility that the PIE of K^+ observed in the rat heart might result from a release of catecholamines from nerve endings, possibly from the K^+-induced depolarization of sympathetic nerve terminals. To eliminate the effect of catecholamines, atrial muscle preparations were pre-treated with 5 μM propranolol and 2 μM phentolamine. The effectiveness of the α and β adrenergic blockade is shown in Figure 3b. The action of tyramine, an agent which causes the release of endogenous catecholamine from the nerve endings, was totally eliminated at these concentrations of propranolol and phentolamine. As shown in Figure 3a, α and β adrenergic blockade did not diminish the PIE of K^+.

To further demonstrate that endogenous catecholamines were not responsible for the K^+-induced PIE, rats were treated with reserpine which has been shown to almost deplete the catecholamine content of cardiac tissue (Akera et al., 1979). Reserpine (5 mg/kg) was injected intraperitoneally about 18 hours prior to sacrifice of the animal. Atrial muscle preparations obtained from reserpinized rats were equilibrated in a K-H buffer containing 3.5 mM K^+. When $[K^+]_o$ was

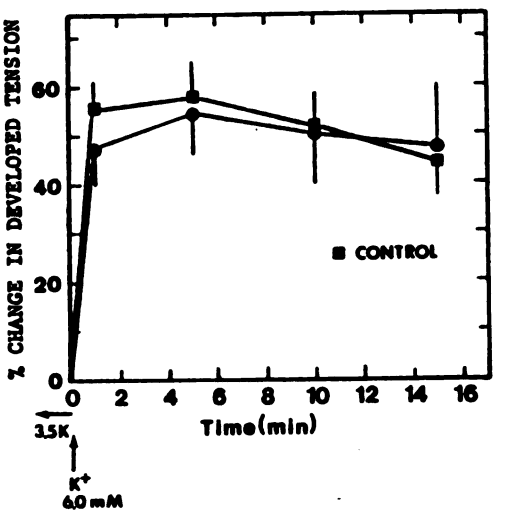

Figure 3a

Figure 3a. Effects of phentolamine and propranolol on the K^+-induced PIE in the rat heart at 30°C. Left atrial muscle preparations of rat were electrically stimulated at 1.5 Hz in a K-H buffer containing 3.5 mM K^+. After a 45-60-min equilibration period, 2 uM phentolamine and 5 uM propranolol was added. K^+ was elevated to 9.5 mM 30 min later at time zero. Changes in developed tension were expressed as percentages of the contractile force observed before elevation of K^+. Vertical lines indicate standard error of the mean. Each point represents 7 to 16 experiments.

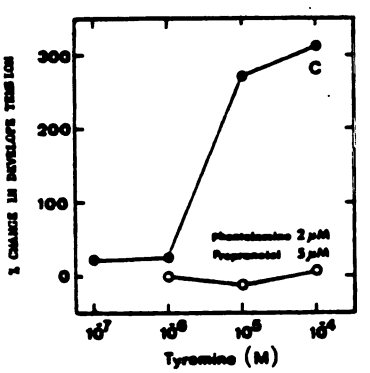

Figure 3b

Figure 3b. Effectiveness of the adrenergic blockade by phentolamine and propranolol on the electrically stimulated left atrial muscle preparations of rat. Conditions were the same as in Figure 3a. Phentolamine (final concentration 2 μM) and propranolol (final concentration 5 μM) was added 30 mins before addition of tyramine. Changes in developed tension were expressed as percentages of contractile force observed before the addition of tyramine. Each point represents the mean of 2 experiments.

raised to 9.5 mM, there was a marked PIE which was of similar magnitude as that observed in control preparations (Figure 4).

The results clearly demonstrate that K^+ induces a reversible positive inotropic effect in the rat heart muscle. This response is quite different from that observed in an other species such as the guinea-pig heart. The K^+-induced PIE observed in the rat heart is unlikely to be caused by catecholamines released from nerve terminals.

B. Frequency Dependency of the K^+-induced Positive Inotropic Effect

It is generally accepted that a low frequency of stimulation favors utilization of the intracellular Ca^{2+} pool whereas a high frequency of stimulation favors utilization of the extracellular Ca^{2+} pool (Allen et al., 1976). Depending on the pool of Ca^{2+} that is activated by K^+, changing the stimulation rate may increase or decrease the magnitude of the K^+-induced PIE. As reported earlier, K^+ induced a 60-80% increase in developed tension when stimulation frequency was 1.5 Hz. However, at 0.5 Hz, K^+ (9.5 mM) induced about a 140% increase in developed tension which was significantly larger than the increase observed at 1.5 Hz (Figure 5). It should be pointed out that the change in frequency of stimulation from 0.5 to 1.5 Hz does not increase the developed tension in the rat heart, in contrast to effects observed in other mammalian species. The developed tension observed at 0.5 Hz in a medium containing 3.5 mM K^+ was 0.35 ± 0.02 g (N=4) and that at 1.5 Hz was 0.35 ± 0.04 g (N=7). After the addition of 6 mM K^+ (final concentration 9.5 mM), the developed tension increased to a maximum value of 0.85 ± 0.04 g and 0.54 ± 0.07 g at 0.5 Hz and 1.5 Hz, respectively. It should be noted that the development of the K^+-induced PIE was delayed when

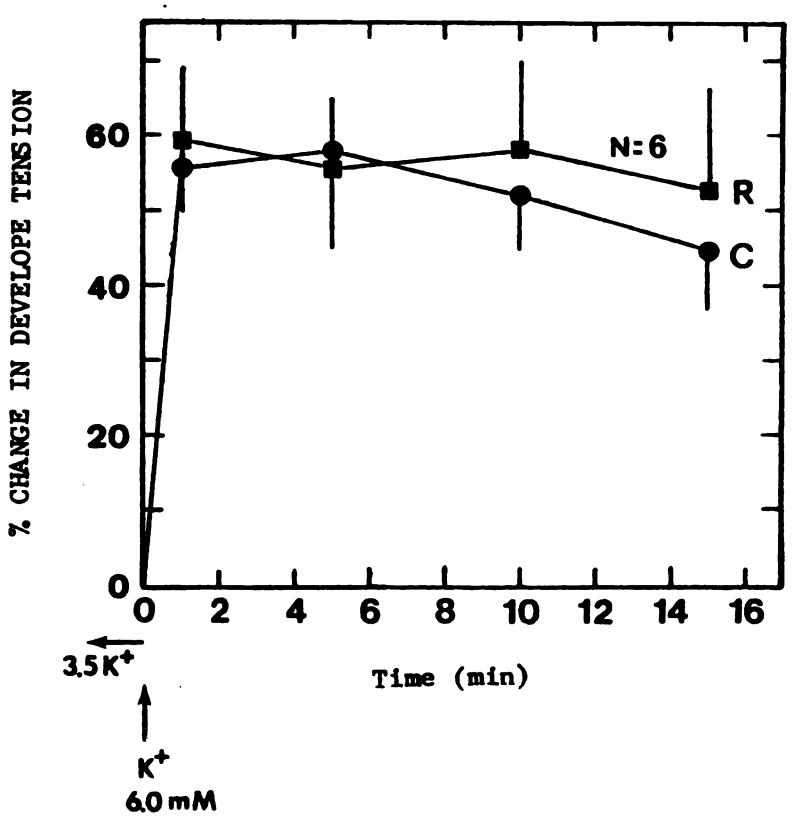

Figure 4

Figure 4. Effect of reserpine treatment on the K^+-induced PIE. Rats were injected with reserpine (5 mg/kg i.p.) 18 to 24 hrs before sacrifice. Left atrial muscle preparations from the rats were electrically stimulated at 1.5 Hz at 30°C. K^+ concentration was elevated to 9.5 mM at time zero. Vertical lines indicate standard error of the mean. Each point represents the mean of 6 experiments.

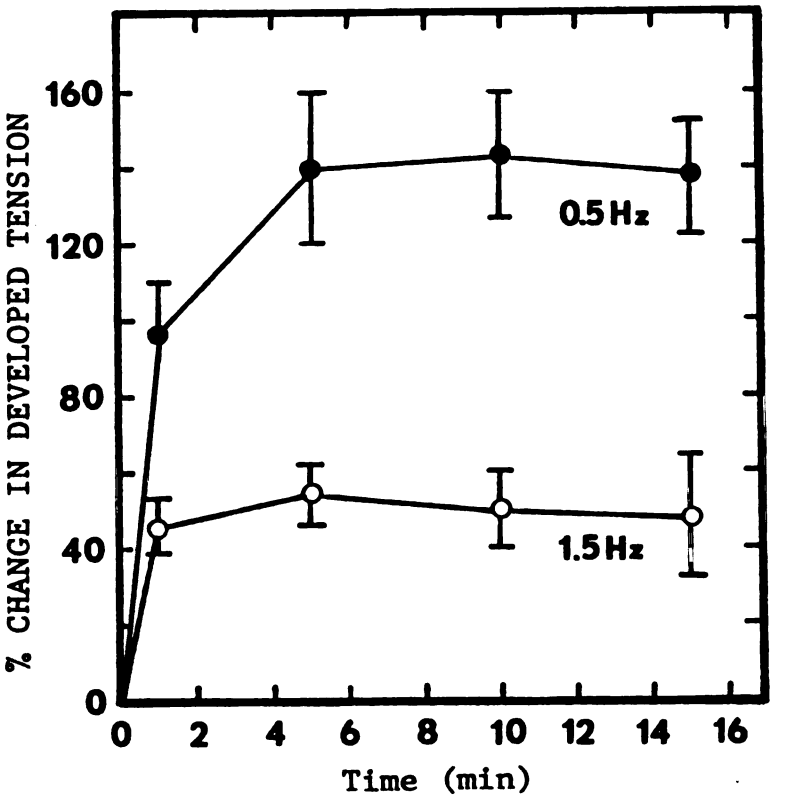

Figure 5

Figure 5. Effects of stimulation frequency on the K^+-induced PIE in left atrial muscle preparations of rat at 30°C. Conditions were the same as in Figure 3a. Phentolamine (2 μM) and propranolol (5 μM) were added 30 mins before elevation of K^+ to 9.5 mM. Atria were stimulated either at 1.5 Hz (○) or 0.5 Hz (●). Changes in developed tension were expressed as percentages of contractile force observed before the elevation of K^+. Vertical lines indicate standard error of the mean. Each point represents the mean of 4 to 7 experiments.

stimulation frequency was further increased. As shown in Figure 6, when the stimulation frequency was increased to 3 Hz, the K^+-induced PIE did not reach a maximum until 11 mins after the addition of K^+. At 3 Hz, the sustained PIE of K^+ was preceded by a transient PIE observed at 1 min. When the stimulation frequency was 0.5 Hz or 1.5 Hz, the maximum PIE was observed within 5 mins without an initial transient increase and subsequent decrease in the developed tension before reaching a steady PIE.

Thus, the K^+-induced PIE was greater at a lower stimulation frequency. Under a high frequency of stimulation (3 Hz), the magnitude of PIE was reduced and the onset rate of the PIE was delayed.

C. Activation of Na,K-ATPase by K^+

The decrease in developed tension which is normally observed when the extracellular K^+ concentration is elevated in isolated heart muscle preparations obtained from several mammalian species has been explained by a K^+-induced stimulation of the Na pump. If this explanation is correct, the paradoxical PIE of K^+ observed in rat atrial muscle preparations may suggest a lack of Na pump stimulation by K^+ in this species. It may be possible that half-maximal activation of the Na pump in the rat heart occurs at a higher or lower K^+ concentration compared to other species. Therefore, changes in $[K^+]_o$ within the physiological range may not have a significant effect on Na pump activity. To evaluate this possibility, the effect of several concentrations of K^+ to activate isolated Na,K-ATPase from rat and guinea-pig heart was examined in the presence of 100 mM Na^+. The enzyme activity observed at different K^+ concentration were analyzed from Hill plots. As shown in

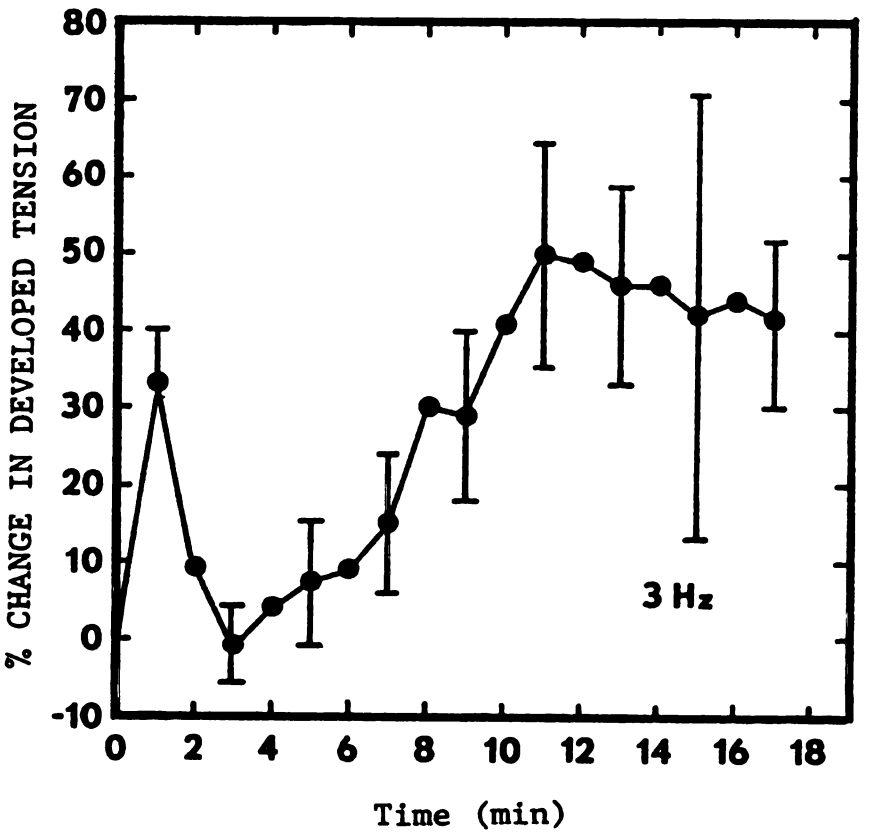

Figure 6

Figure 6. Effects of high stimulation frequency on the K^+-induced PIE in left atrial muscle preparations of rat at 30°C. Conditions were the same as in Figure 5, except stimulation frequency was at 3 Hz. Changes in developed tension were expressed as percentages of contractile force observed before the elevation of K^+. Vertical lines indicate standard error of the mean. Each point represents the mean of 4 experiments.

Figure 7, the Km value for K^+ activation of rat heart Na,K-ATPase was 1.05 mM which was slightly higher but not substantially different from the Km value of 0.78 mM for K^+ stimulation of guinea-pig heart Na,K-ATPase. Thus, there was no marked difference in the K^+-induced activation of Na,K-ATPase in rat and guinea-pig myocardium to explain the difference in the effect of K^+ on the force of contraction.

D. Biphasic Response of Rat Heart to K^+

Since the lowest $[K^+]_o$ tested (3.5 mM) was about 3 times higher than the Km value obtained from isolated enzyme preparations, there is a possibility that the Na pump of the rat heart may not have been sufficiently inhibited to cause accumulation of $[Na^+]_i$ when atrial muscle preparations were incubated in a medium containing 3-9 mM K^+. In guinea-pig left atrial preparations, extrasystoles occurred if $[K^+]_o$ was lower than 4 mM. This suggests, although it does not prove, that the Na pump might be inhibited sufficiently to cause accumulation of $[Na^+]_i$ in atrial muscle preparations of the guinea-pig heart when incubated in a medium containing 4 mM K^+. A larger Na^+ load during each excitation of the guinea-pig atrial muscle may explain the different responses of rat and guinea-pig atrial muscle to $[K^+]_o$ depletion.

To ascertain that $[K^+]_o$ was sufficiently low to inhibit the Na pump of rat atrial muscle preparations, the K^+ concentration in the K-H buffer was further reduced and the force of contraction and stability of the atrial muscle preparations were examined. The rat heart preparations remained stable at $[K^+]_o$ as low as 1 mM during a 60-min equilibration period without showing signs of extrasystoles (data not shown). In subsequent experiments atrial muscle preparations of rat was

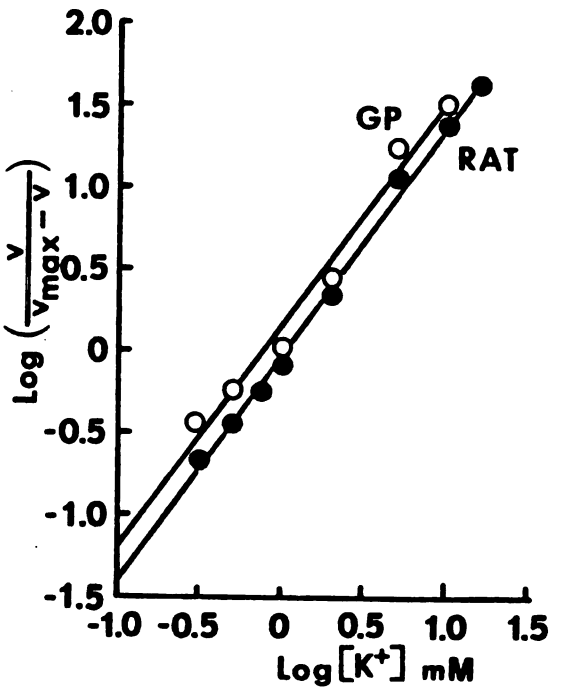

Figure 7

Figure 7. Activation of the Na,K-ATPase by K^+. Partially purified Na,K-ATPase preparations from either guinea-pig (○) or rat heart (●) (0.1 mg protein/ml) was incubated in a medium containing 0.5 mM Tris-ATP, 5 mM $MgCl_2$, 100 mM NaCl, and 50 mM Tris-HCl buffer. After a 10 min pre-incubation period in the absence of KCl, ATPase reaction was started by the addition of various concentrations of KCl as indicated. Velocity of the enzyme activities over a 10 min period were plotted against K^+ concentration in a Hill plot. The Hill coefficient (n) for guinea-pig heart enzyme was 1.36 and K_m for K^+ activation was 0.78 mM. For the rat heart enzyme, n=1.42 and K_m=1.05 mM.

first equilibrated for 60-min in a K-H buffer containing 1 mM K^+. $[K^+]_o$ in the K-H buffer was then increased stepwise to 2, 3, 4, 6 and 9 mM. The developed tension decreased to about 40% of the contractile force observed at 1 mM $[K^+]_o$ when $[K^+]_o$ was raised to 3 mM (Figure 8). However, developed tension increased thereafter when $[K^+]_o$ was further elevated.

E. K^+ Concentration in Rat Plasma

Due to the biphasic nature of the effect of K^+ on the force of contraction observed in rat atrial muscle preparations, the K^+ concentration in the plasma was examined to assess the level of K^+ to which rat myocardium is subjected physiologically. An examination of the existing data suggested that the rat plasma K^+ concentration may be lower than in other species (Songu-Mize et al., 1983; 1984). However, there are also reports indicating that the K^+ concentration in the rat plasma may be equal to, or higher than, that in other species (Cutilletta et al., 1977; Huot et al., 1983).

In order to clarify these differences, K concentration in rat plasma was analyzed. Rats were first anesthetized with ether. Blood was withdrawn from the descending aorta into a heparinized syringe. K^+ concentration of the clear plasma after removal of red cells was assayed using a Na^+/K^+ analyzer with ion selective electrodes (Orian 1020). The K^+ concentration in the plasma was 3.20 ± 0.11 mM (N=5), which is considerably lower than the plasma K^+ concentrations of other species.

F. Neonatal Rat Heart and the K^+-induced PIR

Langer et al. (1975b) have reported that papillary muscle of

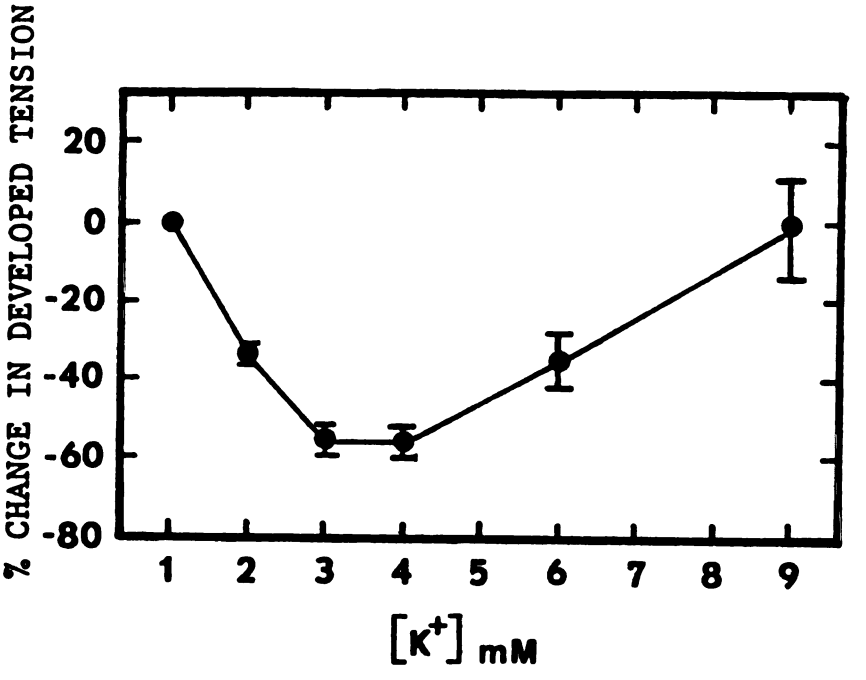

Figure 8

Figure 8. Effects of K^+ on the developed tension of left atrial muscle preparations of rat stimulated at 0.5 Hz at 30°C. Atria were equilibrated for 60 mins in a K-H buffer containing 1.0 mM K^+. K^+ was added to increase $[K^+]_o$ in the K-H buffer to 2, 3, 4, 6 and 9 mM. Changes in developed tension were expressed as percentage of contractile force observed at 1 mM K^+. Vertical lines indicate standard error of the mean. Each point represents the mean of 6 experiments.

new-born rats behaved very much like adult cardiac tissue of other species; i.e. papillary muscle of new-born rats exhibited positive force-frequency relationship, an action potential with a prominent plateau phase and normal sensitivity to cardiac glycosides. To examine whether the basic properties of cardiac muscle responsible for the lack of the above characteristics are also related to the K^+-induced PIE observed in the adult rat heart, the effect of K^+ on the force of contraction was examined in atrial muscle preparations obtained from neonatal rats. If the K^+-induced PIE is somehow related to the unique negative force-frequency relationship of the rat heart, then K^+ ion would not be expected to have a PIE on the neonatal rat heart. The results of our experiments, however, indicated that atrial muscle preparations of the one and three day old rat have a slight negative force-frequency relationship between 0.25 to 1 Hz as observed in the adult rat myocardium (Figure 9). Langer et al. (1975b) reported that papillary muscles of rats at the similar age (5 days to 21 days) have a positive force frequency relationship. Due to the small size of the neonatal rat heart, papillary muscle could not be obtained and was not studied in the present study. In atrial muscle preparations, K^+ induced a biphasic inotropic response in the new-born rats (Figure 10).

Rat heart muscle has been reported to exhibit a positive force-frequency relationship when $[Ca^{2+}]_o$ was lowered (Forester and Mainwood, 1974). In our hands, however, rat atrial muscle preparations maintained the negative force-frequency relationship when $[Ca^{2+}]_o$ was reduced to 0.5 mM (data not shown). Therefore, attempts to study the relationship between the force-frequency relationship and the K^+-induced PIE was not successful.

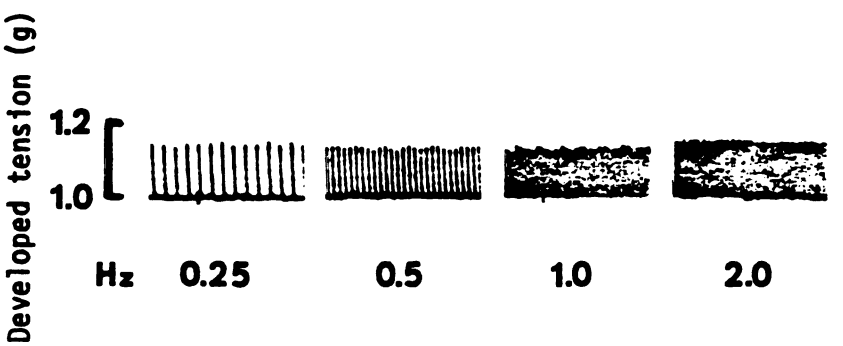

Figure 9

Figure 9. A typical tracing of the effects of stimulation frequency on the developed tension of left atrial muscle preparations of 3 day old neonatal rat at 30°C. After a 60 min equilibration period in a K-H buffer containing 3.5 mM K^+, stimulation frequency was increased from 0.25 Hz to 0.5, 1.0 and then 2.0 Hz.

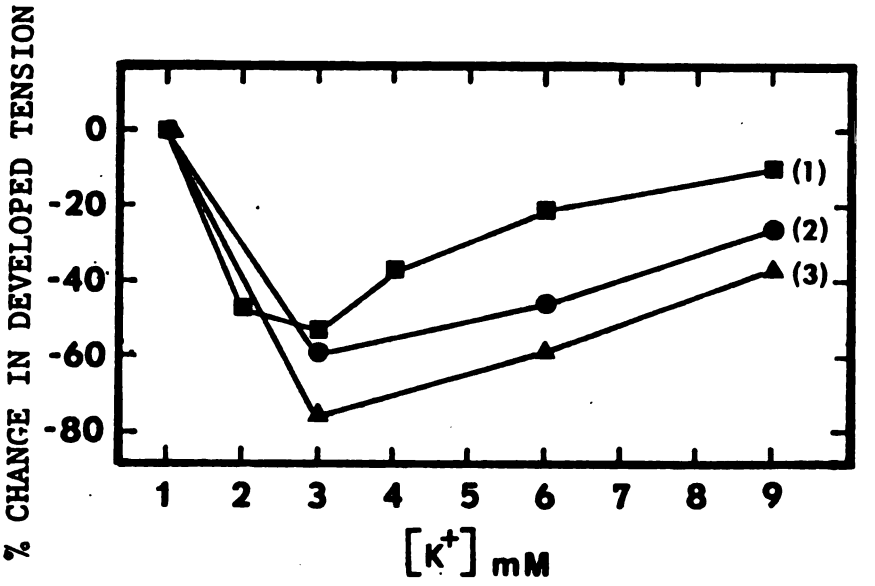

Figure 10

Figure 10. Effects of K^+ on the developed tension of left atrial muscle preparations of neonatal rats stimulated at 0.5 Hz at 30°C. After a 45 min equilibration period in a K-H buffer containing 1 mM K^+, K^+concentration was raised to 2, 3, 4, 6 and then to 9 mM. Numbers in parentheseis indicates the age of the neonatal rats. Changes in developed tension were expressed as percentage of contractile force observed at 1 mM K^+. Each point represents 1 experiment.

G. Temperature Dependency of the K^+-induced PIE

Many biochemical reactions are slowed when the temperature is lowered and among them is the turnover rate of the Na pump. Because the K^+-induced PIE may involve the Na pump, a change in the Na pump rate by lowering the temperature may influence the K^+ effect. In addition, it is important to examine the inotropic effect of K^+ at a lower temperature because voltage clamp experiments that will be described later were performed at room temperature (25-26°C). As shown in Figure 11, K^+ increased the developed tension to about 155% of the control value when stimulation frequency was at 0.5 Hz. However, K^+ induced only a transient PIE when the atrial muscle preparations were stimulated at 1.5 Hz. Therefore, these data seem to suggest a possible relationship between the Na pump and the K^+-induced PIE; the PIE of K^+ seems to be attenuated at a lower Na pump turnover rate, although other possibilities, such as temperature effect on ionic conductance (Cohen et al., 1976; Cota et al., 1983), cannot be ruled out from these experiments alone.

H. Effect of Increasing Na^+ Influx

The above experiment suggests that the K^+-induced PIE may be related to $[Na^+]_i$ because a lower turnover rate of the Na pump would probably lead to accumulation of $[Na^+]_i$. In order to increase the $[Na^+]_i$, left atrial muscle preparations of rat were pretreated with 0.1, 0.5 or 1 μM veratridine. Veratridine increases $[Na^+]_i$ by increasing the Na influx across the sarcolemmal membrane (Sperelakis and Pappano, 1968; Romey et al., 1980). Veratridine alone caused a dose-dependent PIE

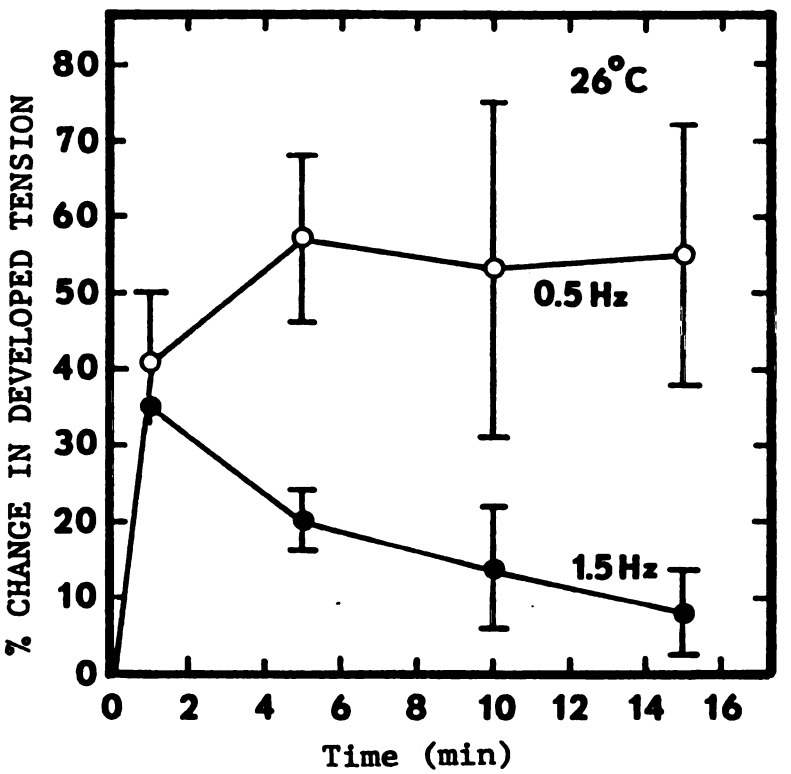

Figure 11

Figure 11. Effects of incubation temperature on the K^+-induced PIE in left atrial muscle preparations of rat. Conditions were the same as in Figure 5, except the incubation temperature was lowered to 26°C. Vertical lines indicate standard error of the mean. Each point represents the mean of 4 experiments.

(data not shown). In the presence of 0.1 and 0.5 μM veratridine, K^+ still produced a biphasic inotropic response when $[K^+]_o$ was increased from 1 μM to 3, 4, 6, and 9 μM (Figure 12). However, the PIE of K^+ was smaller in the presence of 0.5 μM veratridine than that observed in its absence or in the presence of 0.1 μM veratridine. In the presence of 1 uM veratridine only a monotonic negative inotropic effect was observed when $[K^+]_o$ was increased from 1 mM to 3, 6, and 9 mM. Therefore, the K^+-induced PIE was either attenuated or totally eliminated by veratridine depending on the concentration of veratridine used. These data suggests that the relative Na^+ load plays an important role in the expression of the K^+-induced PIE.

I. Electrophysiological Studies

1. Transmembrane Potentials in Multicellular Preparations

It is well documented that when $[K^+]_o$ of cardiac tissue is elevated, there is not only a depolarization of the resting membrane potential, but also a shortening of the APD (Noble, 1976; Cleeman, 1981). In view of the paradoxical inotropic response of rat heart to K^+ ion, it is therefore important to study the K^+-induced changes in the action potential parameters. Action potential was recorded from intact left atrial muscle preparations of rat heart with a KCl-filled glass microelectrode. The preparations were electrically stimulated at 1.5 Hz and equilibrated at 30°C. Under control conditions where the $[K^+]_o$ in the medium was 3.5 mM, the resting membrane potential (RMP) was -78.5 ± 1.4 mV. The time for 20%, 50% and 90% repolarization (T_{20}, T_{50} and T_{90} respectively) was 3.6 ± 0.7 ms, 7.7 ± 0.6 ms and 41.1 ± 1.1 ms respectively (Table 1). After control recordings were

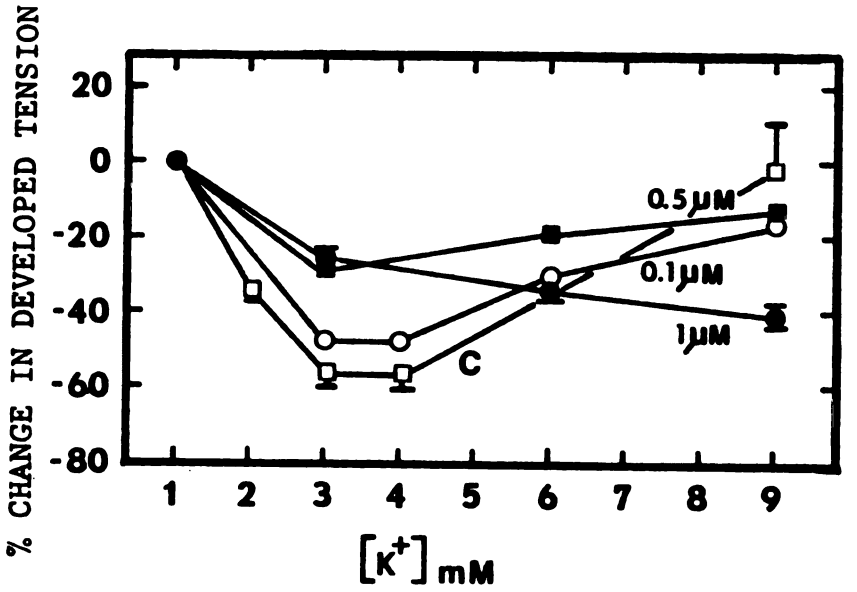

Figure 12

Figure 12. Effects of veratridine on the K^+-induced PIE in left atrial muscle preparations of rat stimulated at 0.5 Hz at 30°C. Conditions were the same as in Figure 7, except veratridine (0.1, 0.5 or 1 μM) was added 17-22 mins before elevation of K^+ to 3, 4, 6, and 9 mM. Phentolamine (2 μM) and propranolol (5 μM) was added before the addition of veratridine. Changes in developed tension were expressed as percentages of contractile force observed before the elevation of K^+. Vertical lines indicate standard error of the mean. Each point represents the mean of 2 to 4 experiments. C: control without veratridine.

TABLE 1

Effect of K^+ on the Transmembrane Potentials of Rat Heart

Left atrial muscle preparations of rat were equilibrated in a K-H buffer containing 3.5 mM K^+ at 30°C and stimulated at 1.5 Hz. Transmembrane potentials were recorded as described in Methods. After the recording of the control experiment, K^+ was superfused into the chamber at time zero to increase the $[K^+]_o$ to a preset concentration of about 9 mM.

	RMP(mV)	T_{20}(ms)[1]	T_{50}(ms)[2]	T_{90}(ms)[3]
control	-78.5±1.4	3.6±0.7	7.7±0.6	41.1±1.1
1 min	-73.1±2.0*	3.6±0.7	7.8±0.6	38.3±1.1*
5 min	-61.9±1.2*	5.9±1.9	9.8±1.7	32.3±1.5*
10 min	-59.0+2.1*	5.5+1.1*	10.0+0.9*	35.9+2.4

* Significantly different from the corresponding control values ($P<0.05$).
[1] Action potential duration at the level of 20% repolarization.
[2] Action potential duration at the level of 50% repolarization.
[3] Action potential duration at the level of 90% repolarization.
Values are the mean ± S.E.M. of 8 experiments.

obtained, K^+ concentration of the medium was increased by adding KCl solution to the superfusion medium. After 10 min of K^+ superfusion, the extracellular K^+concentration increased to about 9 mM (data not shown). The RMP was depolarized to -59.0±2.1 mV and the T_{20} and T_{50} increased significantly to 5.5±1.1 ms and 10.0±0.9 ms, respectively. The T_{90} shortened significantly at 1 min and 5 min after infusion of K^+. However, the T_{90} at 10 min after K^+ infusion is not significantly different from control. These results indicate that the response of rat atrial muscle to an elevation of K^+ with respect to action potential configuration is different from that observed in most other species (Carmeliet and Vereecke, 1979).

In another series of experiments, these action potential parameters were measured at 0.5 Hz and 1.5 Hz. As shown earlier, at 26oC K^+ induced a much-sustained PIE when stimulation frequency was at the 0.5 Hz while only a smaller transient PIE could be induced at 1.5 Hz. Therefore, it is possible to examine whether the observed changes in electrophysiological parameters, such as the increase in APD, could be the sole factor in determining the PIE of K^+.

At 0.5 Hz, RMP changed from -88±2 mV to -71±2 mV when $[K^+]_o$ was increased from 3.5 mM to 9.5 mM. At 1.5 Hz, RMP changed from -83±2 mV to -69±2 mV (Table 2). The reason for the greater depolarization by K^+ at 0.5 Hz than at 1.5 Hz is not clear, although accumulation of K^+ in the extracellular cleft under higher stimulation may play a role (Boyett and Jewell, 1980). The T_{20}, T_{50} and T_{90} increased significantly from 12±1 ms to 16±1 ms, from 25±1 ms to 21±2 ms, and from 79±4 ms to 90±5 ms, respectively, at 1.5 Hz. At 0.5 Hz, only T_{50} was increased significantly from 24±1 ms to 26±1 ms.

TABLE 2

Effects of Stimulation Frequency and $[K^+]_o$ on the Transmembrane Potentials of Rat Heart

Left atrial muscle preparations of rat were equilibrated in a K-H buffer containing 3.5 mM or 9.5 mM K+ at 26oC and stimulated at the indicated frequency. Transmembrane potentials were recorded as described in Methods. Either the stimulation frequency or the $[K^+]_o$ was then changed to a new value as indicated and transmembrane potentials were again recorded. Transmembrane potentials under four different conditions were recorded from each impalement.

Hz	$[K^+]_o$(mM)	RMP(mV)	T20(ms)	T50(ms)	T90(ms)
1.5	3.5	-83±2	12±0.6	25±1	79±4
0.5	3.5	-88±2	12±0.7	24±1	87±5
0.5	9.5	-71±2	12±0.6	26±1	94±3
1.5	9.5	-69+2	16+1.0	31+2	90+5

* Two values connected by the vertical bar are significantly different ($p<0.05$)
Values are the mean ±S.E.M. of 6 experiments.

Therefore, the APD was more prolonged by K^+ at 1.5 Hz than at 0.5 Hz. However, the degree of membrane depolarization was slightly greater when the stimulation frequency was lower.

2. Electrophysiological Studies in Isolated Myocytes

a. Morphology of the Isolated Myocytes

Myocytes of rat heart were isolated from atrial and ventricular muscle by the enzymatic dispersion procedure described in the methods. Figure 13 shows the typical morphology of the isolated myocytes. The rod-shaped cells, which constituted from 5% to 50% of the isolated cell population, were considered to be viable. Some of these cells initially showed spontaneous contractions; however, they usually became quiescent when suspended in oxygenated K-H buffer. The ventricular myocytes have a dimension of about 100 μm x 20 μm, while the atrial myocytes has a dimension of about 70 x 15 μm estimated from a calibrated eyepiece on the microscope. The ventricular cells have clear striations while the atrial cells have less distinct striations. These cells usually remain quiescent for a 5 to 6 hour period and retain their rod-shape appearance even in the presence of 1.8 mM $Ca^{2+}$and thus can be regarded as Ca^{2+} tolerant (Powell and Twist, 1976).

b. Voltage Clamp Experiment with Isolated Myocytes

The prolongation of APD caused by an elevation of $[K^+]_o$ to 9.5 mM (Table 1) suggests that high K^+ may have altered the ionic current(s) during the repolarization phase of the action potential. A prolongation of the APD would allow a greater influx of Ca^{2+} and thus may explain the K^+-induced paradoxical PIE.

To test this hypothesis, voltage clamp experiments were performed using isolated single rat myocytes with a single suction

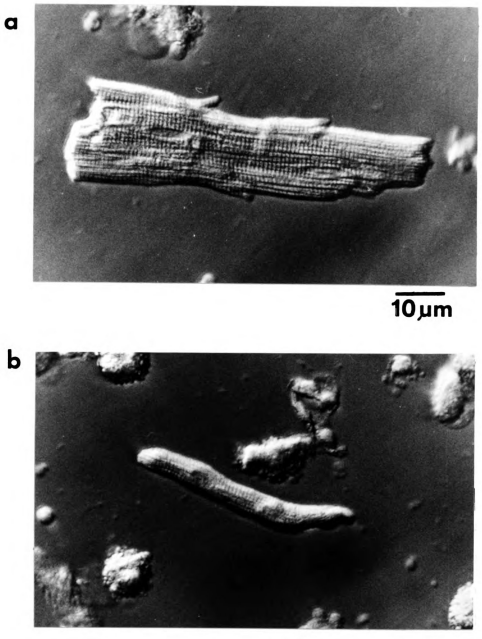

Figure 13

Figure 13. Morphology of the isolated myocytes of rat heart. (a) ventricular cell (b) atrial cell

pipette method described by Hume and Giles (1983). This technique eliminates the inherent problems of the multicellular preparations such as the inability to inject current uniformly (Johnson and Liebermann, 1971; Attwell and Cohen, 1977; Noble, 1979), and depletion and/or accumulation of ions in a restricted extracellular space (see Hume and Giles, 1981).

c. Electrical Properties of the Isolated Myocytes

Only the quiescent, rod-shaped cells were used for the present electrophysiological studies. Upon a successful impalement, a stable RMP, which is within the range of -75 mV to -90 mV with 3.5 mM K^+, could be recorded. In cases where impalement failed, no stable RMP could be established and cells very often showed spontaneous contractions.

At the resting membrane potential, input resistance (R_{in}) was calculated from steady-state hyperpolarization produced by small, inward current pulses of 100 ms duration (Table 3). The estimated R_{in} ranges from 31 to 84 megohms with an average of 48.3±6.7 megohms for ventricular cells and 77.0±13.7 megohms for atrial cells. The reason for the differences in the R_{in} between atrial cells and ventricular cells is probably due to the smaller size of atrial cells which would have lower surface to volume ratio. Time constant for these cells ranges from 4 to 8ms, with an average of 6.9±0.6 ms for ventricular cells and 6.5±0.5 ms for atrial cells. The R_{in} observed in the present study is similar to that reported by Brown *etal*. (1981). However, the time constant is significantly shorter from that reported by the above investigators (17.6±0.45 ms).

Action potential was elicited by passing a depolarizing

TABLE 3
Electrical Properties of Isolated Myocytes

	Ventricular cell	Atrial cell
Input resistance	48.3±6.7 mΩ(8)	77.0±13.7 mΩ(4)
Cell membrane time constant	6.9±0.6 ms (8)	6.5±0.5 ms (4)
Resting membrane potential	-87±1 mV (11)	-86±2 mV (9)
Action potential duration[1]	74±7 ms (11)	90±13 ms (9)
Action potential amplitude	121+3 mV (11))	123+5 mV (9)

$[K^+]_o$ = 3.5 mM temperature: 25-26°C
[1] Action potential duration at the level of 90% repolarization.
Values are the mean ± S.E.M. Numbers in parenthesis indicate the number of experiments.

current of 1 ms duration into the cell through the recording electrode. Figure 14 shows a typical action potential recorded from a rat ventricular cell. The arrow indicates the stimulus artifact. The RMP, APD and APM of the ventricular and atrial cells are shown in Table 3. Both rat ventricular and atrial cells have very similar action potential characteristics. Although atrial cells seem to have a longer APD, the difference is not statistically significant. The action potential parameters of these isolated cells are comparable to that of multicellular preparations (compare Table 2 and Table 3). Because these results were obtained at room temperature (24-26oC), the APD is considerably longer, probably due to the slower kinetics of ion fluxes at the lower temperatures.

d. Ionic Currents Underlying the action potential of the Rat Heart

To understand the ionic currents underlying the action potential of isolated myocytes, voltage clamp experiments were performed. In some cases, the action potential parameters were first measured and then the electronics was switched to the voltage clamp mode. In cases where cells were treated with TTX to eliminate the Na^+ current, the voltage clamp was switched on after a stable RMP was established without taking action potential recordings. Figure 15a shows a typical current tracing when RMP was clamped at -90 mV and then suddenly depolarized to various potentials. The very brief outward current (outward current points upward and inward current points downward) is due to capacitive discharge. The fast inward current is probably composed of a Na^+current and a Ca^{2+}current and has been studied extensively (Reuter, 1979). A transient outward current which decays

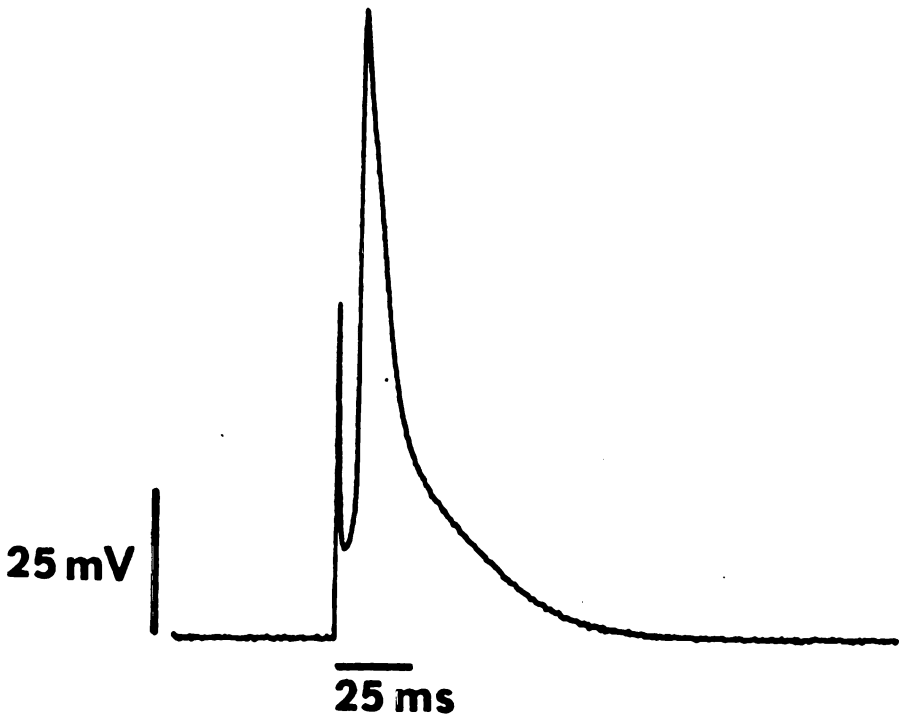

Figure 14

Figure 14. Transmembrane potential from isolated myocytes of rat ventricle. Action potential was elicited by a 1 ms current pulse applied intracellularly at 0.25 Hz. Cells were suspended in a K-H buffer containing 3.5 mM K^+ at room temperature. Rest membrane potential was -87 mV. Time and voltage calibration is as shown. Arrow indicates stimulus artifact. Typical tracing from 1 of 11 cells studied.

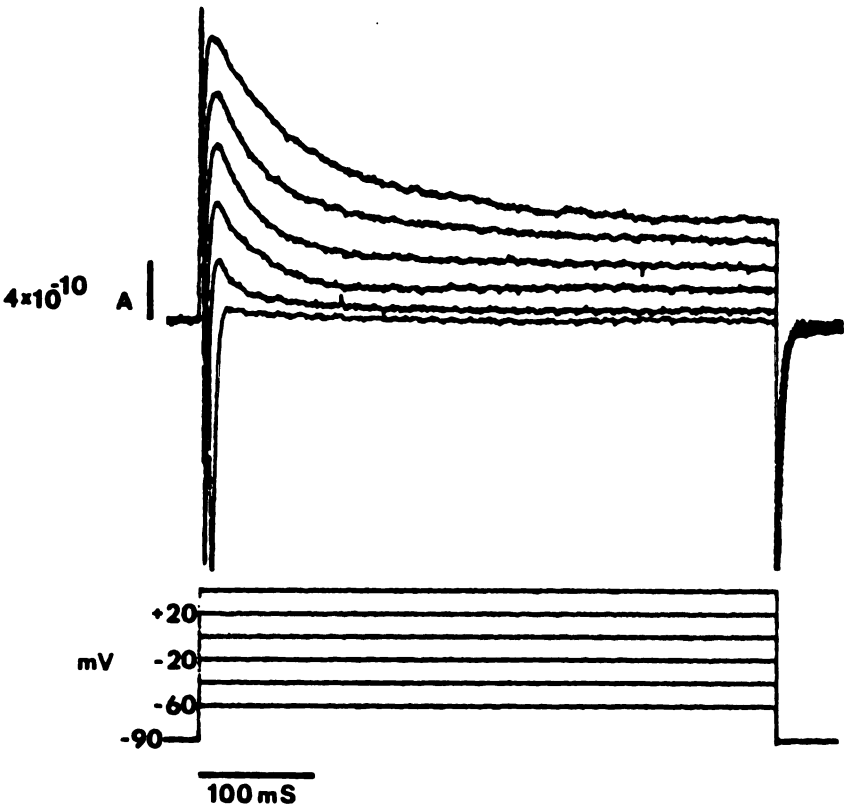

Figure 15a

Figure 15a. Membrane currents in the isolated rat ventricular myocytes. The membrane currents were elicited by 500 ms duration voltage steps from a holding potential of -90 mV to -60, -40, -20, 0, +20, +40 mV. Lower traces: stimultaneous voltage recordings.

with time during the voltage-clamp is also prominent. The magnitude of this outward current increases with more positive membrane potentials. Because it is well known that the APD of rat is very short and may thus be expected to have a very small Ca^{2+}current, it was of interest to examine how much of the Ca^{2+} current contributes to the total inward current. TTX (30 μM), a specific inhibitor of the fast Na^{+}current (Narahashi et al., 1964; Dudel et al., 1967; Narahashi, 1974), was superfused into the cell chamber. The holding potential was clamped at -50 mV to additionally assure inactivation of the fast Na^{+} channel. As can be seen in Figure 15b, in the presence of 30 μM TTX, a concentration that would be sufficient to block the Na^{+} current in cardiac muscle (Narahashi, 1974), and with a reduced holding potential, an inward current persisted. This inward current is likely to be the Ca^{2+} current via slow channels because it can be eliminated by Ca^{2+} blockers such as Cd^{2+} (data not shown).

The background current-voltage relationship was also studied. Membrane potential was held at -90 mV and was depolarized or hyperpolarized to various potentials with 500 ms pulses. Currents at the end of this clamp step were plotted against the test pulse voltage (Figure 16a and b). In the presence of 3.5 mM K^{+}, the time independent background current exhibited the inward-going rectification as reported for cardiac tissues of other species (Noble, 1965; Noble, 1979; Noble and Tsien, 1968). Higher K^{+} (9.5 mM) did not cause an outward shift of the I-V curve either in the atrial (Figure 16a) or in the ventricular muscle cell (Figure 16b). Such a shift is often seen in the background current-voltage relation in other species studied (Noble, 1976; Cleeman, 1981) and has been called the " cross-over" phenomenon.

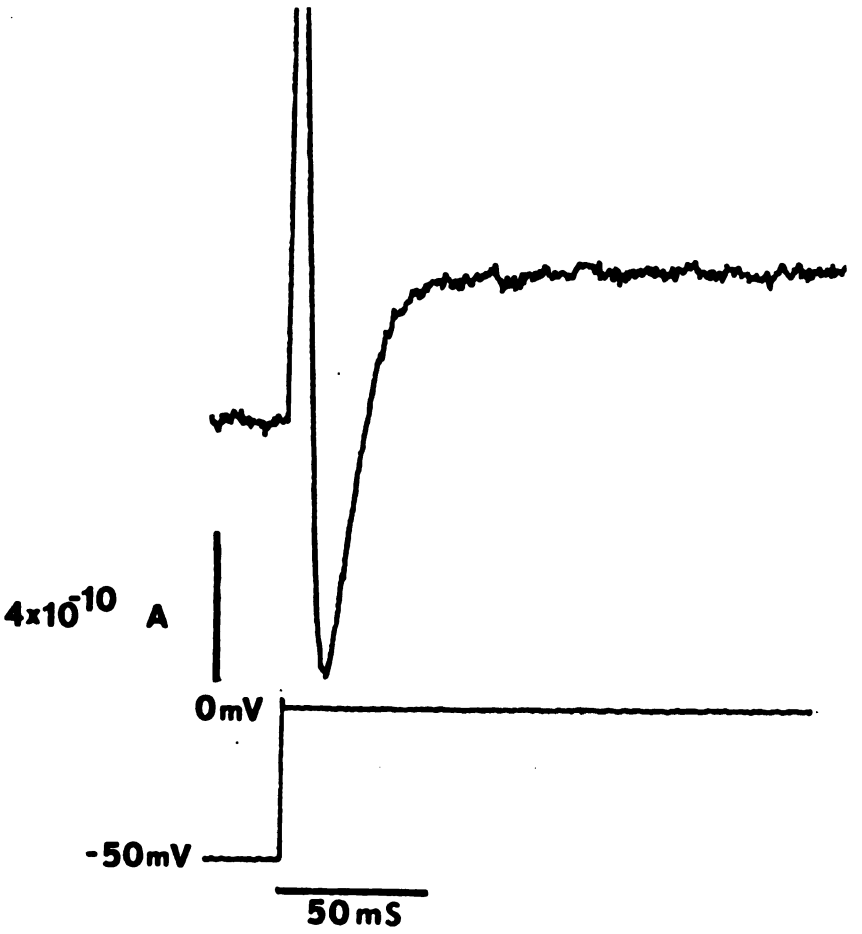

Figure 15b

Figure 15b. Slow inward current in isolated ventricular myocytes of rat. The inward current was elicited by a 50 mV voltage-clamp step of 500 ms duration from a holding potential of -50 mV. TTX (30 μM) was present. Lower trace: stimultaneous voltage recording.

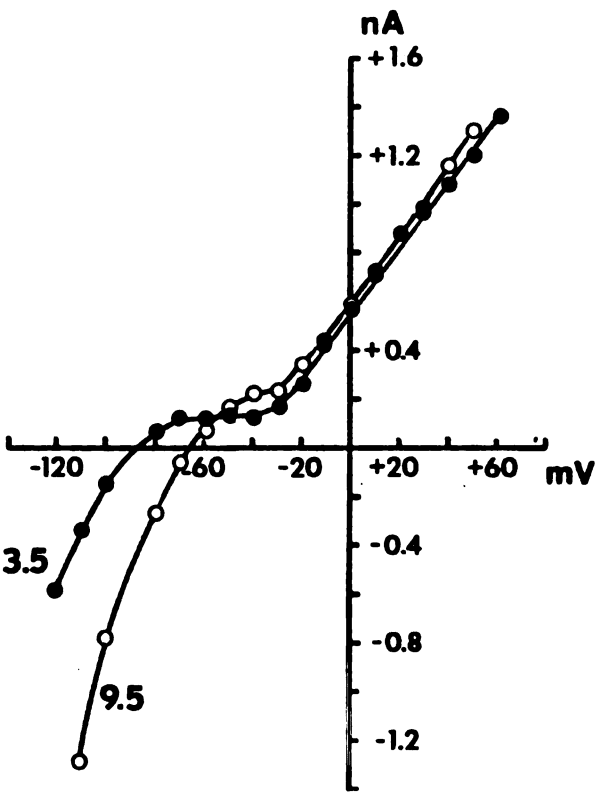

Figure 16a

Figure 16a. Effects of K^+ concentration on the background current of isolated atrial myocytes of rat. Currents were elicited by a 500 ms voltage-clamp step from a holding potential of -90 mV. I-V relationship at the end of the 500 ms clamp step was measured. Myocytes were first superfused with a K-H buffer containing 3.5 mM K^+ (●). K^+concentration was then increased to 9.5 mM (○).

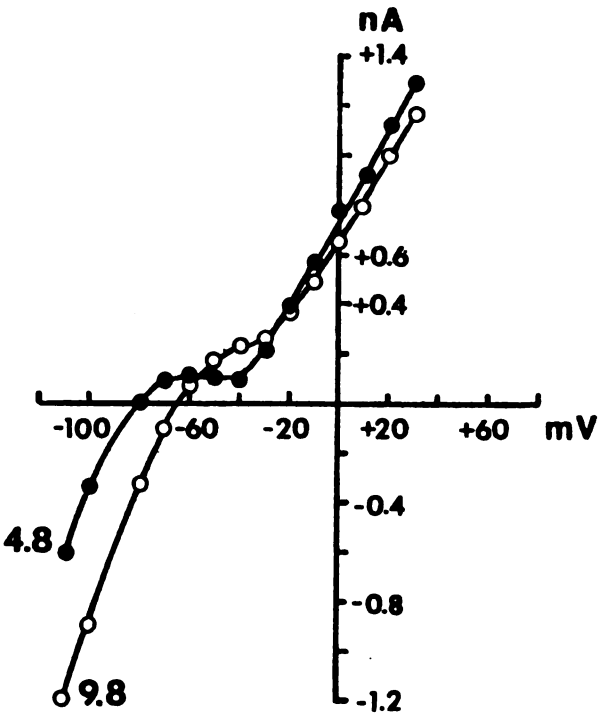

Figure 16b

Figure 16b. Effects of K^+ concentration on the background current of isolated ventricular myocytes of rat. Conditions were the same as in Figure 16a, except the myocytes were first superfused with a K-H buffer containing 4.8 mM K^+ (○) and then increased to 9.8 mM K^+ (●).

e. A Transient Outward Current

The current record in Figure 15a reveals a novel early transient outward current (I_{EO}). Similar early outward currents have been reported in cardiac Purkinje fibers from calf (Peper and Trautwein, 1968; Kenyen and Gibbons, 1979; Siegelbaum and Tsien, 1980). Such an outward current might be responsible for the short duration of action potential observed in the rat heart. In addition, the presence of an early outward current and its alteration by K^+, if observed, might possibily explain the paradoxical effect of K^+ on the rat heart. Therefore, voltage clamp experiments were performed to further characterize the I_{EO}.

In order to examine the current-voltage relationship of I_{EO}, the membrane potential was held at -90 mV and subsequently changed abruptly to various membrane potentials by 500 ms current pulses. The magnitude of the I_{EO} increased with a more positive membrane potential (Figure 17). The magnitude of the I_{EO} was defined either as the peak early outward current minus the holding current at -90 mV or as the peak early outward current minus the current at the end of the pulse. The latter definition may be a better representation of the magnitude of the early outward current because the contribution of the background outward current is subtracted out. Therefore, in the following experiments the early outward current will be so defined. Contaminations contributed by I_{Na} and I_{Ca} was not subtracted from the I_{EO} in these experiments. To examine whether this outward current inactivates, voltage-induced inactivation of this current was studied in isolated atrial and ventricular myocytes. After a 2 sec conditioning pulse at potentials between -130 mV and +50 mV, the membrane potential was changed to -20

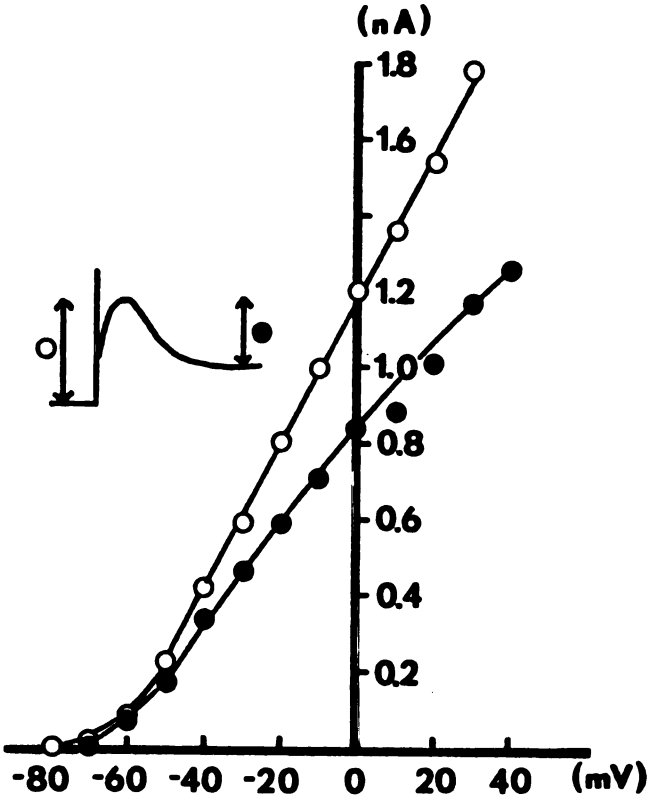

Figure 17

Figure 17. Current-voltage relationship of the transient outward current (I_{EO}) in isolated atrial myocytes of rat. The currents were elicited by a 500 ms duration voltage steps from a holding potential of -90 mV. The magnitude of the early outward current was defined either as the peak early outward current minus the holding current (O) or as the peak early outward current minus the background time-independent outward current at the end of the 500 ms voltage step(●).

mV, a potential at which the I_{EO} was sufficiently activated, for 500 ms and returned to the holding potential of -90 mV. Figure 18a illustrates the current records of such an experiment with isolated atrial myocytes. As the conditioning pulse became less negative, the magnitude of the I_{EO} became progressively diminished. The inward current that preceded the I_{EO} is probably a combination of the fast Na^+ current and the slow inward current. As the I_{EO} became inactivated, the inward current became more evident. The activation of the I_{EO} became faster as the conditioning potential was more negative. These data plus those from ventricular myocytes were replotted in Figure 18b in which the I_{EO} was normalized by setting the maximum I_{EO} elicited at the holding potential of -130 mV at 100%. In each case, the I_{EO} was completely inactivated at a holding potential between -60 to -50 mV. The half inactivation occurred at about -70 mV.

Siegelbaum and Tsien (1980) reported the presence of a Ca^{2+}-activated transient outward current in calf Purkinje fibers. Removal of $[Ca^{2+}]_o$ or blockade of slow inward current greatly diminished the outward current. To test whether the I_{EO} observed in the rat myocytes was also Ca^{2+}-activated, $[Ca^{2+}]_o$ was reduced to 0.2 mM by changing the superfusing K-H solution. As shown in Figure 19, the I_{EO} at high and low $[Ca^{2+}]_o$ were almost superimposible, suggesting that this current is probably not Ca^{2+}-induced.

The transient outward current observed in sheep Purkinje fibers has been shown to be depressed by 4-aminopyridine (Kenyan and Gibbons, 1979), an agent which blocks K^+ currents in nerve and skeletal muscle (Pelhate and Pichon, 1974; Gillespie and Hutter, 1975; Schauf *et al.*, 1976; Yeh *et al.*, 1976a, b; Meves and Pichon, 1977). To test

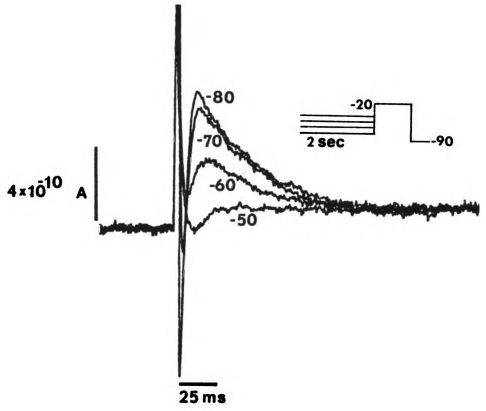

Figure 18a

Figure 18a. Steady state inactivation of the early outward current in isolated atrial myocytes of rat. Conditioning pulses of 2 sec duration from -80 mV to -50 mV was followed by a 500 ms voltage-clamp step to -20 mV. Typical tracing from one of the three myocytes studied.

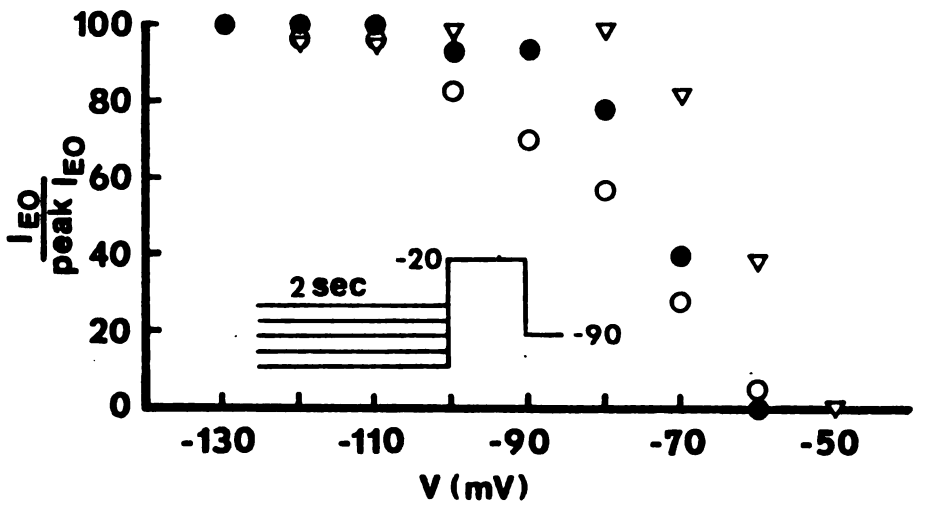

Figure 18b

Figure 18b. Inactivation of the early outward currents in isolated atrial and ventricular myocytes of rat. Conditionds were the same as in Figure 18a except the 2 sec conditioning pre-pulses varied from -130 mV to -50 mV (● ○) Ventricular cells, (▽) atrial cell. Magnitude of I_{EO} observed at a conditioning pulse of -130 mV was set at 100%.

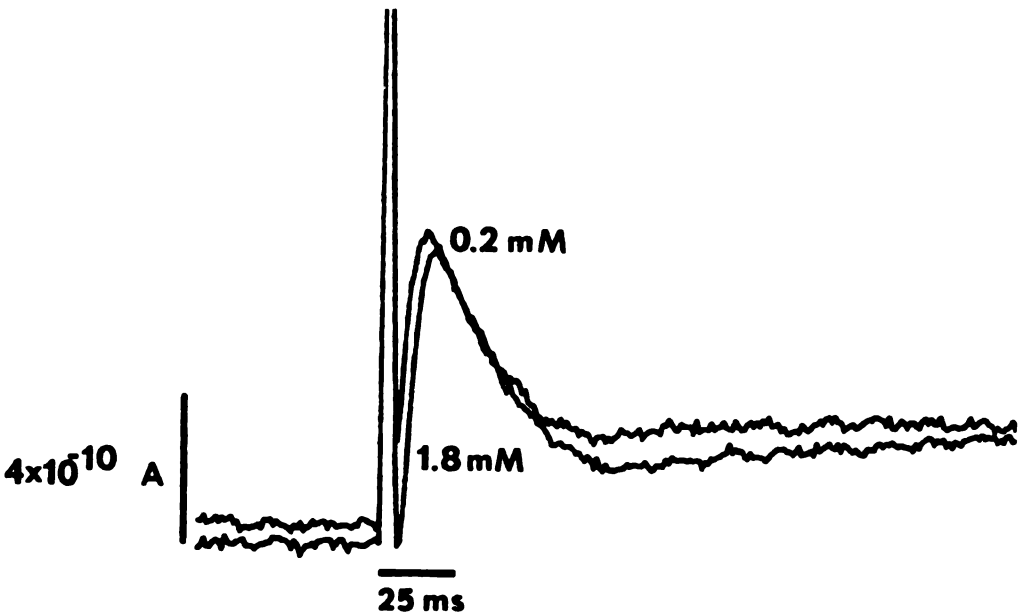

Figure 19

Figure 19. Effect of Ca^{2+} on the transient outward current of isolated rat ventricular myocytes. The outward current was elicited by a 70 mV depolarizing step of 500 ms duration from a holding potential of -90 mV. The K-H buffer contained 3.5 mM K^+ and (a) 1.8 mM Ca^{2+} and (b) 0.2 mM Ca^{2+}.

whether the I_{EO} of rat myocytes is a K^+ current, I_{EO} was compared in the presence and absence of different concentrations of 4-aminopyridine (Figure 20). Cd^{2+} (10 μM) and TTX (30 μM) were also present to reduce the Ca^{2+} current and the fast Na^+ current, respectively. The presence of 3-5 mM 4-aminopyridine totally eliminated the I_{EO} suggesting that the current is most likely a K^+ current. With the blockade of the transient outward current a small inward current, which is likely due to the Ca^{2+} current, became evident. Therefore, I_{EO} is likely to be a K^+ current since 4-aminopyridine totally abolished it.

J. Involvement of I_{EO} in the K^+-induced PIE

From transmembrane potential studies, it is clear that an increase in $[K^+]_o$ from 3.5 to 9.5 mM caused about a 20 mV depolarization (Table 1). It is also clear that the transient outward current was almost totally inactivated when the membrane potential was depolarized from -80 mV to -60 mV (Figure 16). Therefore, it is reasonable to postulate that the K^+-induced PIE may be due to inactivation of the I_{EO} by the depolarizing effect of K^+ ion. Inactivation of the I_{EO} would prolong the APD which in turn could allow greater Ca^{2+} influx. To test this hypothesis, the K^+-induced PIE was studied in the presence and absence of the I_{EO}. As shown earlier (Figure 20), 4-aminopyridine at concentrations from 3-5 mM totally eliminated the I_{EO}. Thus, it provided a valuable tool for the proposed experiments. If 4-aminopyridine abolishes the I_{EO} then it should also prolong the APD. Figure 21 shows the effect of 5 mM 4-aminopyridine on the transmembrane potential of an isolated rat ventricular cell. One minute after the superfusion of 4-aminopyridine, there was a clear prolongation of the

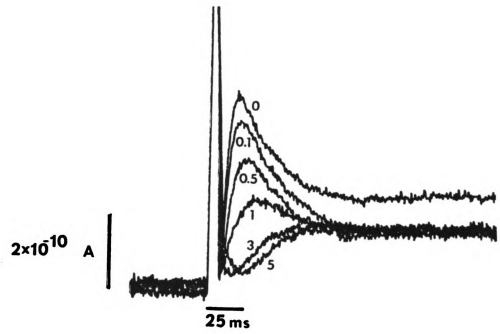

Figure 20

Figure 20. Effect of 4-aminopyridine on the transient outward current of isolated ventricular myocytes of rat. The currents were elicited by a 70 mV depolarizing voltage-clamp step from a holding potential of -90 mV. 4-aminopyridine (0.1, 0.5, 1, 3 and 5 mM) was added at 3 min intervals cummulatively to the superfusing K-H buffer containing 3.5 mM K^+. Typical tracing from one of the three myocytes studied.

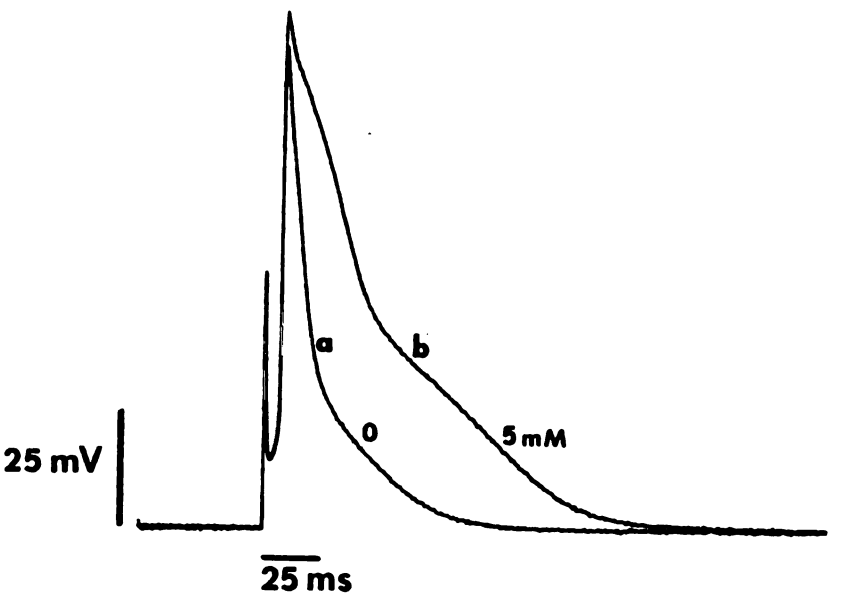

Figure 21

Figure 21. Effect of 4-aminopyridine on the transmembrane potential of isolated ventricular myocytes of rat. Action potential was elicited by a 1 ms depolarizing current pulse at a stimulation frequency of 0.25 Hz. Action potentials before (a) and 10 mins after addition of 5 mM 4-aminopyridine (b).

APD. The prolongation of the APD reached its maximum 5 min after addition of 4-aminopyridine. Similar effect of 4-aminopyridine on the APD has also been reported recently by Mitchell et al. (1984). The force of contraction should also be increased as the result of lengthening of the APD. Figure 22 shows that indeed 4-aminopyridine increased developed tension of rat atrial muscle preparations in a dose dependent manner. A maximum effect was reached at 3-10 mM 4-aminopyridine. The data seem to be consistent with the voltage clamp experiment in which 3-5 mM 4-aminopyridine appeared to significantly reduce the I_{EO}.

Therefore, the inotropic effect of K^+ in the presence of 3 mM 4-aminopyridine was examined. Addition of 6 mM K^+ to elevate the $[K^+]_o$ from 3.5 to 9 mM increased the developed tension from 0.39±0.04 to 0.88±0.07 g (Figure 23). When $[K^+]_o$ was returned to 3.5 mM, the developed tension returned to the control level of 0.39±0.04 g. Addition of 3 mM 4-aminopyridine caused a 74.4% increase in developed tension (0.39±0.4 to 0.68±0.3 g), when the K^+ concentration was raised to 9.5 mM in a medium containing 3 mM 4-aminopyridine, the developed tension increased almost an additional 100% (0.68±0.03 to 1.25±0.07 g). Epinephrine (10^{-5} M) further increased the developed tension indicating that developed tension had not reached the maximum value possible in the presence of 3 mM 4-aminopyridine and 9.5 mM K^+. These results indicate that in the absence of I_{EO}, $K^+$is still capable of producing a large PIE. This suggests that elimination of I_{EO} by K^+ is not the only mechanism for the K^+-induced PIE.

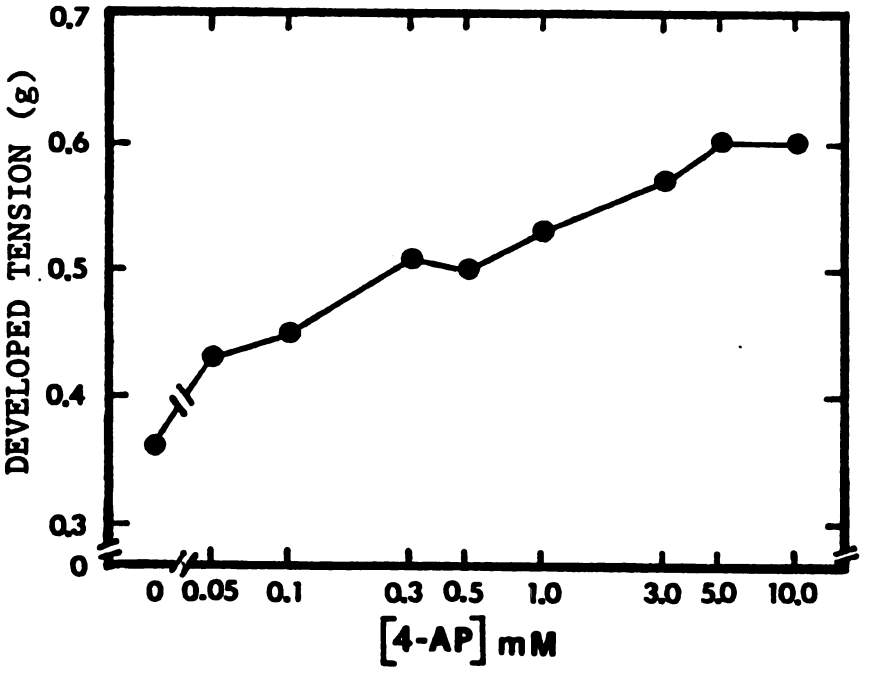

Figure 22

Figure 22. Effects of 4-aminopyridine on the developed tension of rat heart at 30°C. Left atrial muscle preparations of rat heart were stimulated at 0.5 Hz in a K-H buffer containing 3.5 mM K^+. After a 60 min equilibration period, 4-aminopyridine (neutralized with HCl) was added at 15 min intervals. Mean of two experiments.

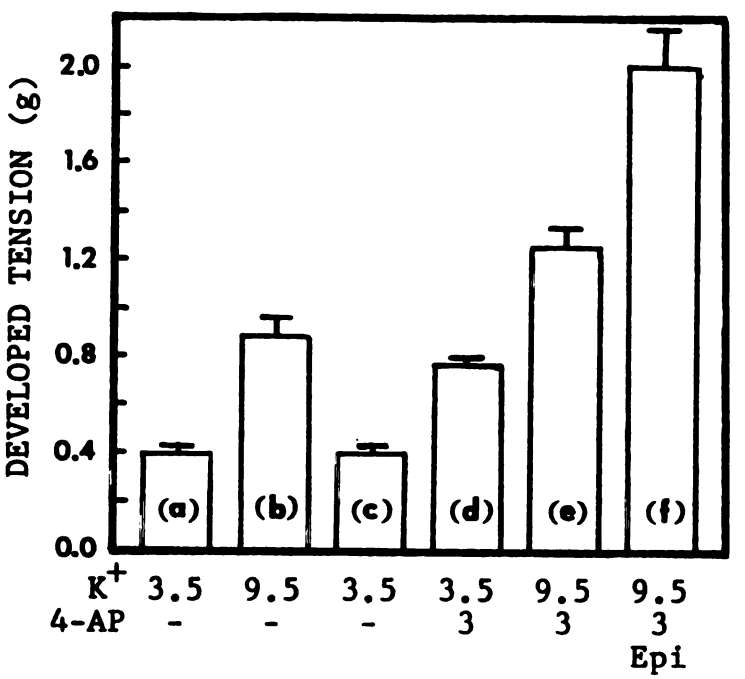

Figure 23

Figure 23. Effect of 4-aminopyridine on the K^+-induced PIE in the rat heart. Left atrial muscle preparations of rat were stimulated at 0.5 Hz at 30°C in a K-H buffer containing 3.5 mM K^+. After a 60 min equilibration period, developed tension was recorded (a). K^+ concentration was raised to 9.5 mM. The developed tension at 5 min after the addition of K^+ was recorded (b). K-H buffer containing 3.5 mM K^+ was quickly washed in to reduce the K^+ concentration to 3.5 mM. After a 15 min equilibration, the developed tension was again recorded (c) and 3 mM 4-aminopyridine was added. After 15 mins, the developed tension was recorded (d) and K^+ contration was raised to 9.5 mM in the presence of 3 mM 4-aminopyridine. The developed tension was recorded 5 mins later (e). Finally, 10 uM epinephrine was added and the developed tension was recorded 10 mins later (f). Phentolamine (2 μM) and propranolol (5 μM) were present during the entire experiment. Vertical lines indicate standard error of the mean. Each bar represents the mean of 4 experiments.

K. Specific Ca^{2+} Pool(s) Involved in the K^+-induced PIE

Under physiological conditions, several pools of Ca^{2+} are involved in contractile activation. Therefore, the specific Ca^{2+} pool involved in the K^+-induced PIE was examined. It is generally accepted that there are at least 2 major sources of Ca^{2+} which contribute to the contraction process. One is the Ca^{2+} present in the extracellular space and the membrane bound Ca^{2+} which constitute the extracellular or superficial source. The other is a sarcoplasmic reticulum (SR) Ca^{2+} pool which constitutes the intracellular pool (Fabiato and Fabiato, 1977; Chapman, 1983; Langer, 1984).

1. The Superficial Ca^{2+} Pool

The superficial Ca^{2+} pool has been postulated to be intimately involved in the excitation-contraction process (Bers et al., 1981; Langer, 1984). To determine whether this Ca^{2+} pool is involved in the K^+-induced PIE, Cd^{2+} was used to deplete the superficial Ca^{2+} pool (Bers and Langer, 1979). After a 90 min incubation with 10 μM Cd^{2+}, the developed tension decreased to about 14% of control force (0.84±0.2 g to 0.12±0.02 g). Addition of K^+ (6 mM) induced about a 120% increase in developed tension (Figure 24). In another series of experiments, the Cd^{2+} concentration was increased to 20 μM. The developed tension was reduced to about 13% of the control force after a 60 min incubation period (0.78±0.13 g to 0.10±0.01 g). Addition of K^+ (6 mM), in this case, induced only a transient PIE. These results suggest that the superficial Ca^{2+} pool, which is likely to be depleted by Cd^{2+} (especially at the higher concentration), may be involved in the K^+-induced PIE.

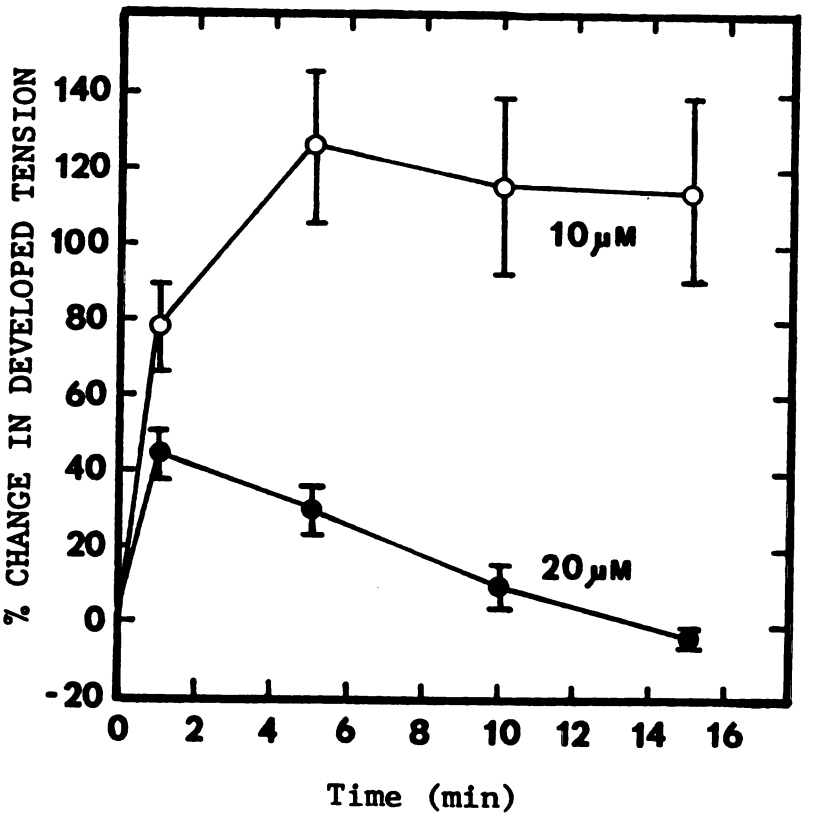

Figure 24

Figure 24. Effects of Cd^{2+} on the PIE of K^+ in rat heart. Left atrial muscle preparations of rat were stimulated at 0.5 Hz at 30°C in a K-H buffer containing 3.5 mM K^+. Cd^{2+} was added after the 45 min equlibration period. After incubation with 10 μM and 20 μM Cd^{2+} for 90 and 60 min, repectively, K^+ concentration was raised to 9.5 mM. Phentolamine (2 μM) and propranolol (5 μM) was present in the experiment with 20 μM Cd^{2+}. Changes in developed tension were expressed as percentage of contractile force observed before the elevation of K^+. Each point represents 5 to 6 experiments.

2. The Intracellular Ca^{2+} Pool

Ryanodine and caffeine have been shown to inhibit Ca^{2+} release from, and Ca^{2+} uptake by, the intracellular SR Ca^{2+} pool with different mechanisms of action (Blayney et al., 1978; Blinks et al., 1972; Jones et al., 1978; Frank and Sleator, 1975). Therefore, the effects of these two agents on theK^+-induced PIE were studied.

Left atrial muscle preparations obtained from rat heart were first treated with 6 nM ryanodine which has been reported to block Ca^{2+} release from the SR (Sutko et al., 1980; Sutko et al., 1979). The developed tension decreased to 8.9+1.2% of the control force after a 90 min incubation in the presence of ryanodine (data not shown). At the same time, post-rest potentiated contraction was totally eliminated; when the preparation was stimulated at 0.5 Hz after a 25-sec quiescent period, the developed tension elicited by the first stimulus was small (Figure 25a). When $[K^+]_o$ was raised from 3.5 mM to 9.5 mM at the end of the 90-min incubation in the presence of ryanodine, K^+ induced a 372.5±55.5% increase in developed tension compared to the force observed just before the addition of K^+ (Figure 25b). This increase is unlikely to be caused by catecholamine release because 5 uM propranolol and 2 uM phentolamine were added 25 mins prior to the addition of K^+.

While the developed tension observed under 0.5 Hz stimulation was enhanced by the addition of 6 mM K^+, the contraction elicited by the first stimulus after rest remained markedly attenuated (Figure 26). Complete recovery of the Ca^{2+} pool which contributes to the post-rest potentiated contraction thus seemed not necessary for the expression of the K^+-induced PIE. These data suggest that the ryanodine sensitive Ca^{2+} pool is not involved in the K^+-induced PIE.

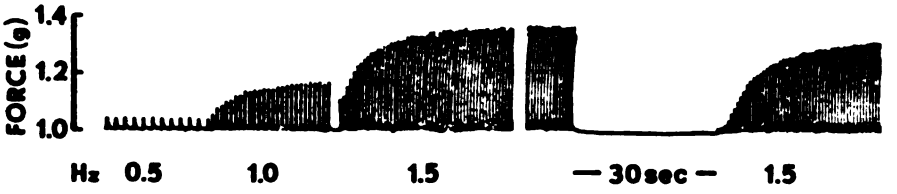

Figure 25a

Figure 25a. A typical tracing of the effects of ryanodine on the force-frequency relationship of rat heart. Left atrial muscle preparations of rat were stimulated at 0.5 Hz at 30°C in a K-H buffer containing 3.5 mM K^+. After a 45 min equilibration period, 6 nM ryanodine (final concentration) was added. The force-frequency relationship was examined by increasing the frequency of stimulation to 1.0 and 1.5 Hz. Post-rest potentiatd contraction was elicited after a 30 sec quiescent period.

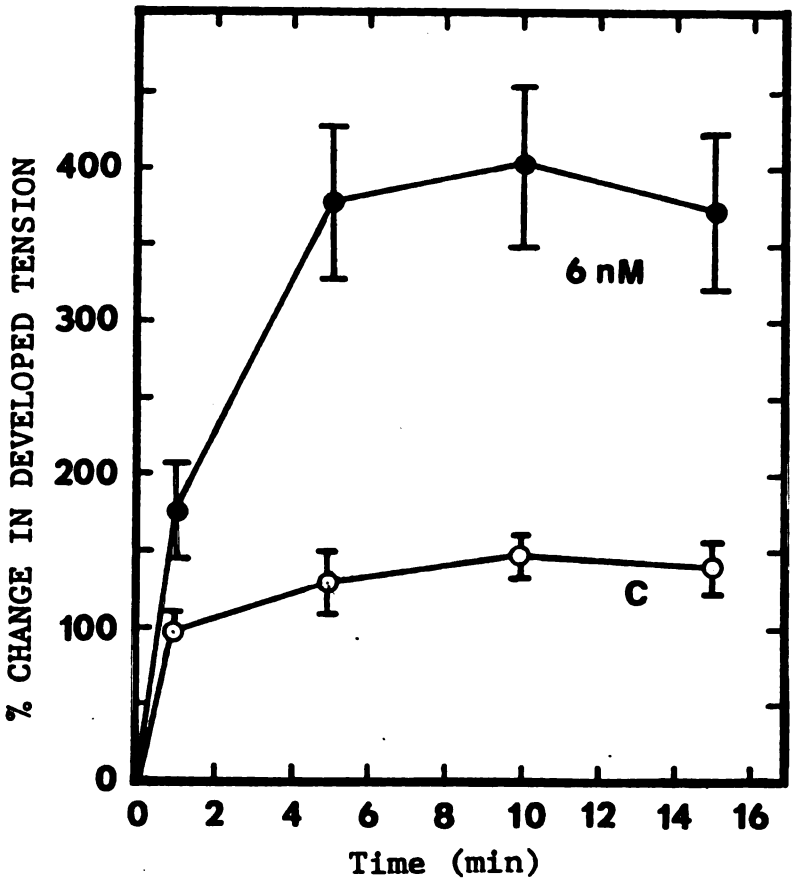

Figure 25b

Figure 25b. Effects of ryanodine on the PIE of K^+ in the rat heart. Left atrial muscle preparation of rat were stimulated at 0.5 Hz at 30°C in a K-H buffer containing 3.5 mM K^+. After a 45 min equilibration, 6 nM (final concentration) ryanodine was added. Phentolamine (2 μM) and propranolol (5 μM) was added 60 min later. K^+ concentration was elevated to 9.5 mM 90 min after the addition of ryanodine. Changes in developed tension were expressed as percentages of contratile force observed before the elevation of K^+. Vertical lines indicate standard error of the mean. C: control without ryanodine. Each point represents 4 to 8 experiments.

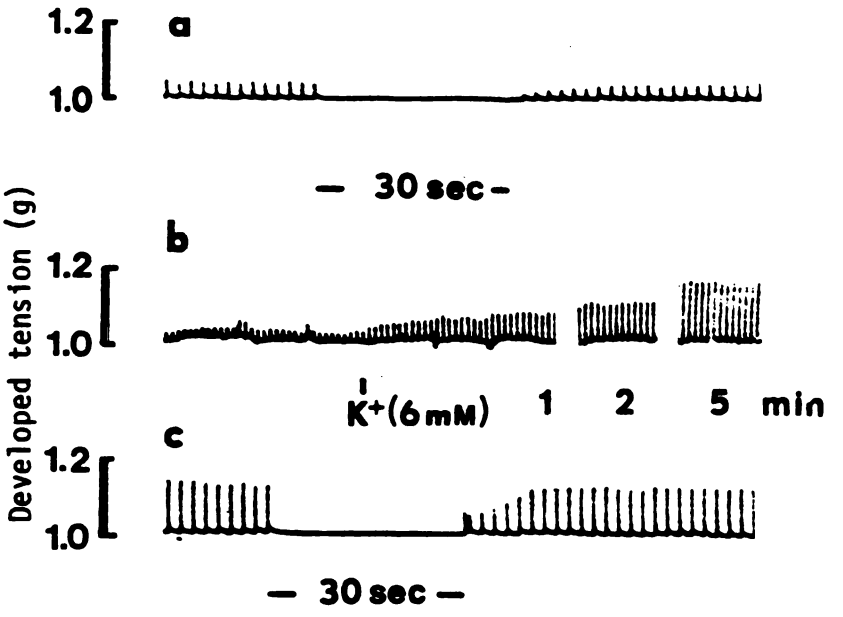

Figure 26

Figure 26. A typical tracing of the effects of ryanodine on the K^+-induced PIE in the left atrial muscle preparations of rat. Experimental conditions were the same as in Fig. 25b. (a) post-rest potentiated contration observed 90 min after incubation with 6 nM ryanodine and before the elevation of K^+. (b) PIE of K^+ in the presence of ryanodine. (c) post-rest potentiated contraction observed after the elevation of K^+.

Caffeine is an agent which has been reported to inhibit Ca^{2+}uptake into the SR and thus alter the function of the SR (Henderson etal., 1974; Blayney et al., 1978). Therefore, caffeine was also tested for its effect on the K^+-induced PIE. Caffeine (3 mM) had very little effect on the developed tension observed under 0.5 Hz stimulation (Figure 27a). This concentration of caffeine abolished the negative force frequency relationship of the rat atrial muscle preparation between 0.25 Hz and 0.5 Hz. Caffeine also abolished the potentiated contraction by the first stimulus after rest; the first contraction after rest had the same magnitude as the following contractions. This effect of caffeine is quite different from that of ryanodine. After ryanodine treatment, the magnitude of the first contraction after rest was very small and gradually returned to steady-state level only after several beats. The K^+-induced PIE was totally eliminated at this concentration of caffeine (Figure 27b). When rat atrial muscle preparations were first treated with ryanodine, caffeine caused a large PIE (data not shown). The positive force-frequency relationship that exists after ryanodine treatment persisted in the presence of 3 mM caffeine (Figure 28a). When K^+ was then added subsequently, a 204% (N=2) increase in developed tension was observed (Figure 28b).

Even though both ryanodine and caffeine are believed to alter the function of the intracellular Ca^{2+} pool, their effects on the K^+-induced PIE were quite different. While caffeine alone totally abolished the PIE of K^+, ryanodine pre-treatment did not diminish the K^+-induced PIE. Quite unexpectedly, when ryanodine was present in addition to caffeine, the K^+-induced PIE persisted. These results seem to be inconsistent with the popular view on the mechanism of action of

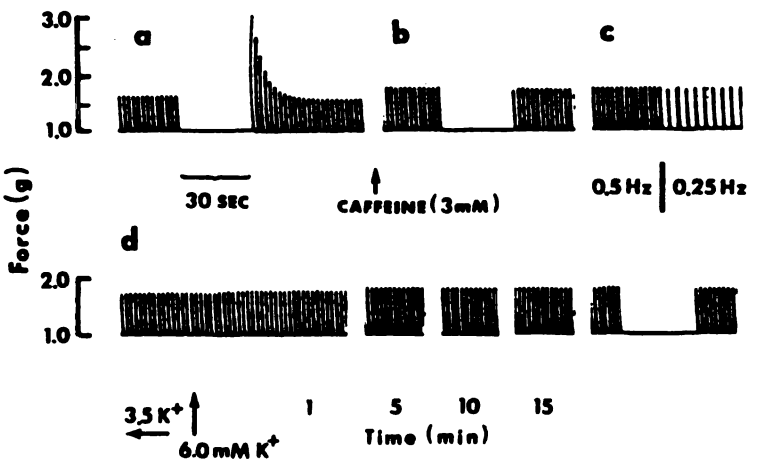

Figure 27a

Figure 27a. A typical tracing of the effects of caffeine on the K^+-induced PIE in the rat heart. Left atrial muscle preparation of rat were stimulated at 0.5 Hz at 30oC in a K-H buffer containing 3.5 mM K^+. After a 60 min equilibration period, post-rest potentiated contration was elicited by a 30 sec quiescent period (a). (b) post-rest potentiated contraction, (c) force-frequency relationship at 0.25 and 0.5 Hz and (d) the PIE effect of K^+ observed after the addition of 3 mM caffeine.

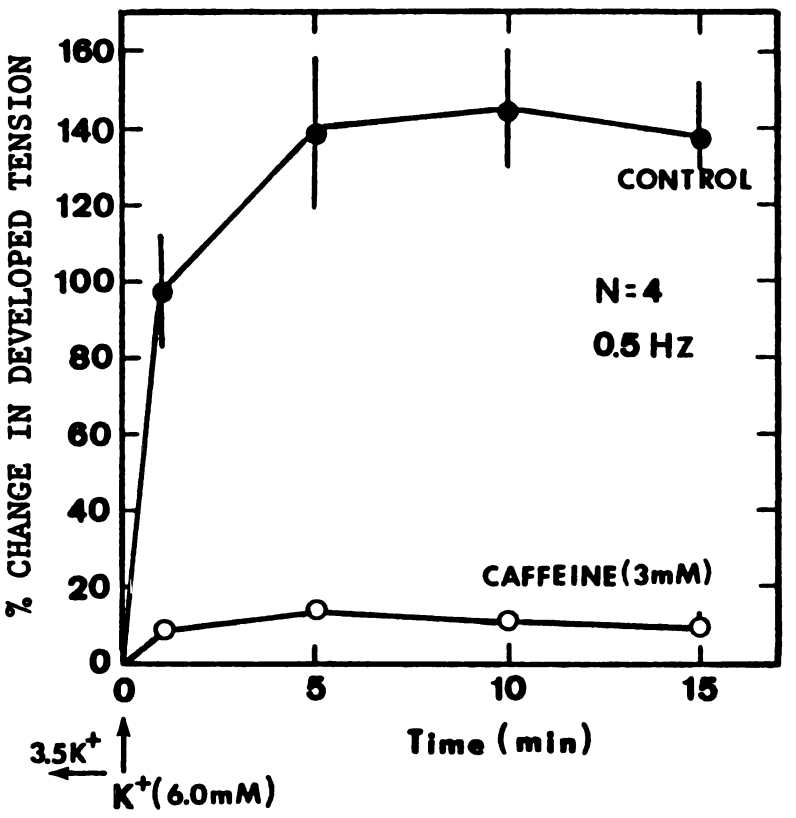

Figure 27b

Figure 27b. Effects of caffeine on the K^+-induced PIE in the left atrial muscle preparation of rat. Conditions were the same as in Fig. 27a. K^+ concentration was elevated to 9.5 mM 35 min after addition of 3 mM caffeine. Changes in developed tension were expressed as percentages of contractile force observed before the elevation of K^+. Vertical lines indicate standard error of the mean. Each point represents the mean of 4 experiments.

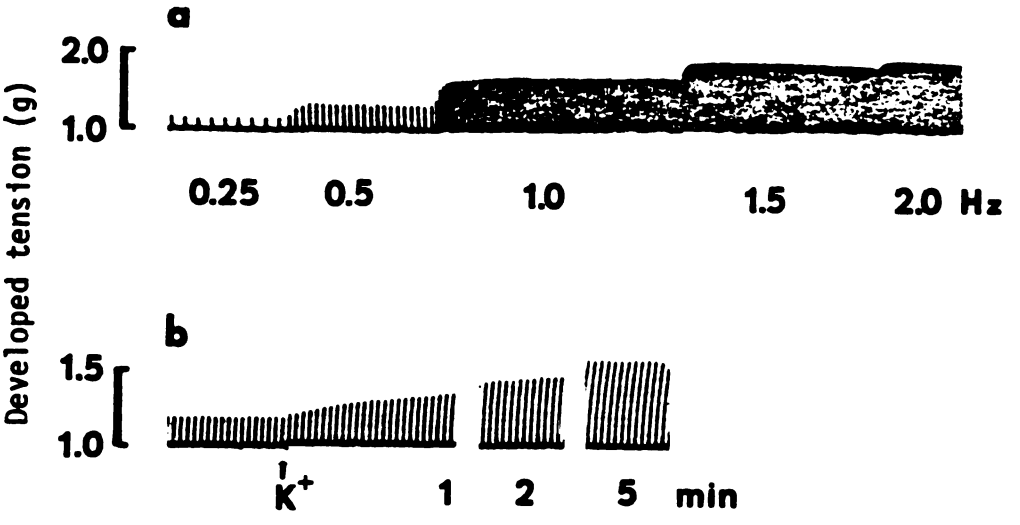

Figure 28a

Figure 28a. A typical tracing showing the effects of caffeine on the ryanodine-induced positive-force frequency relationship in the rat heart. Left atrial muscle preparation of rat was stimulated at 0.5 Hz at 30°C in a K-H buffer containing 3.5 mM K^+. Caffeine (3 mM) was added 60 min after the addition of ryanodine (6 nM). Ninety minutes after the addition of ryanodine, the force-frequency relationship was examined by changing the stimulation frequency from 0.25 to 2 Hz (a) and K^+ concentration was subsequently increased to 9.5 mM (b)

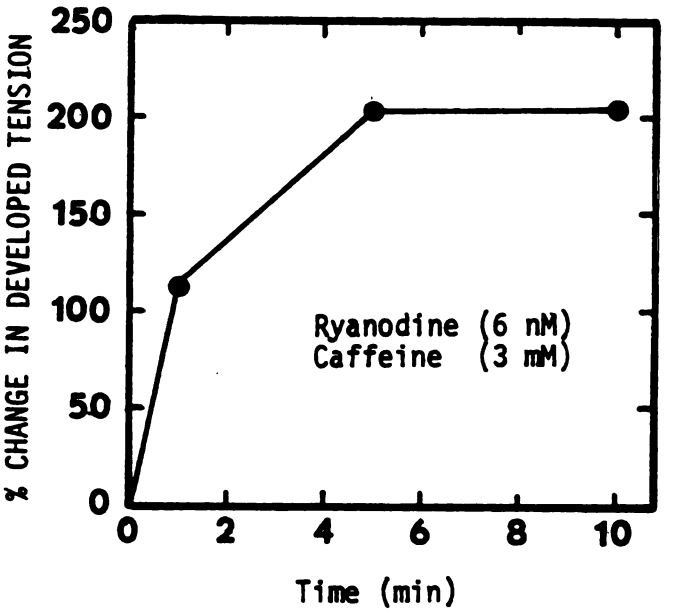

Figure 28b

Figure 28b. Effects of caffeine and ryanodine on the K^+-induced PIE in the left atrial muscle preparations of rat. Experimental conditions were the same as in Figure 28a. K^+ concentration was elevated to 9.5 mM 30 min after the addition of 3 mM caffeine (90 min after the addition of ryanodine). Changes in developed tension were expressed as percentages of contractile force observed before the elevation of K^+. Each point represents the mean of 2 experiments.

ryanodine and caffeine. The significance of these results will be discussed.

L. Biphasic Response of Guinea-Pig Heart to K^+

Results from studies on rat atrial muscle preparations indicated that the PIE of K^+ is frequency dependent. It thus seems possible that under appropriate conditions, i.e. low stimulation frequency, K^+ could also induced a PIE on guinea-pig atrial muscle preparations. To examine this possibility, guinea-pig atrial muscle preparations were stimulated at 0.25 Hz and $[K^+]_o$ concentration was decreased to 1 mM. As pointed out earlier, guinea-pig atrial muscle preparations are unstable in K-H buffer containing $[K^+]_o$ of less than 4 mM. Therefore, in the following experiments, the preparations were first equilibrated in a K-H buffer containing 4.5 mM K^+. A K-H buffer containing 1 mM K^+ was then quickly washed in. The force of contraction of the muscle preparation increased slowly thereafter. At a point where the force seemed to reach a plateau and before arrhythmias occurred (approximately 10-12 min), K^+ was increased stepwise to 3, 6, and 9 mM at 10 min intervals. At 0.25 Hz, developed tension decreased when $[K^+]_o$ was increased to 3 and 6 mM (Figure 29). However, when $[K^+]_o$ was further increased to 9 mM, developed tension was significantly increased. At 0.5 Hz, the negative inotropic effect of K^+ was evident at these concentrations of K^+. Elevation of $[K^+]_o$ to 9 mM only induced a small PIE. Under 1.5 Hz stimulation, $[K^+]_o$ up to 9 mM caused only negative inotropic effects. The negative inotropic effect is thus prominent in the guinea-pig heart muscle. However, under certain conditions, K^+ can also produce a PIE in the guinea-pig heart.

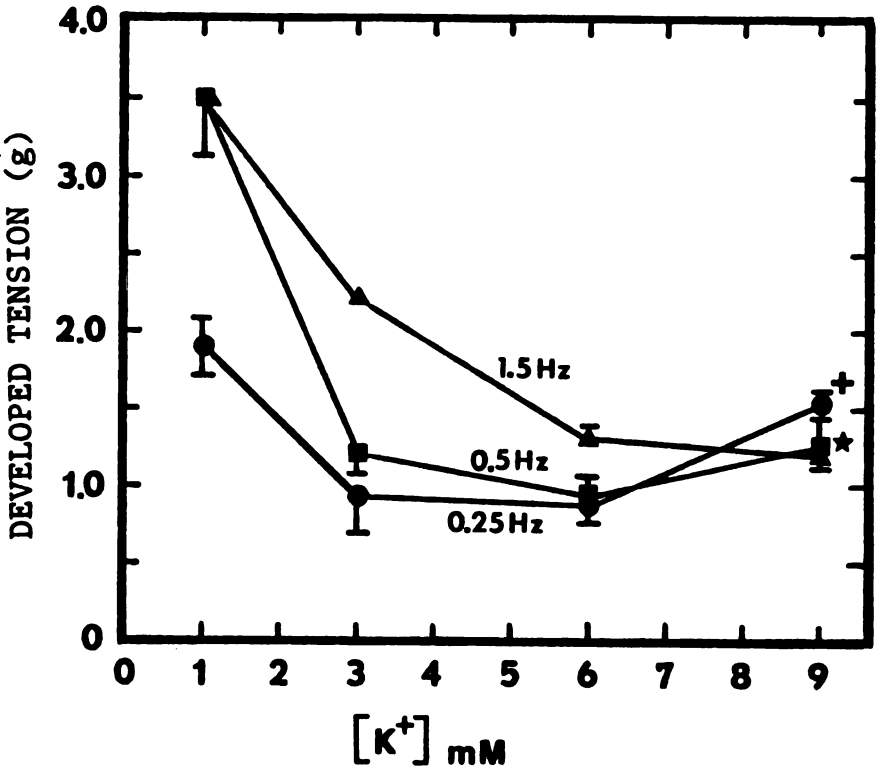

Figure 29

Figure 29. Effects of K^+ on the developed tension of left atrial muscle preparation of guinea pig. See text for experimental protocol. Each atrial preparation was studied at 3 different frequency of stimulation (0.25, 0.5 and 1.5 Hz). Each point represents the mean of 3 experiments. *, +: significantly different from the contractile force observed with 6 mM $[K^+]_o$ ($p<0.02$).

DISCUSSION

A. Activation of the Na Pump by K^+ in Rat Heart

One possible explanation for the different responses of guinea-pig and rat heart toward K^+ is a difference in the activation of the Na pump by K^+. Result of the isolated enzyme study, however, revealed that there is no significant difference in the concentration of K^+ to cause a half-maximal activation of Na,K-ATPase between rat and guinea-pig heart enzymes. The Km value of the rat heart enzyme (1.02 mM) was slightly higher than that of guinea-pig heart Na,K-ATPase (0.78 mM). These values are very close to those observed with the rat brain enzyme (Robinson, 1969). Isolated enzyme studies may not necessarily reflect the K^+-induced activation of the Na pump which takes place in the intact tissue, however; these results do not support the hypothesis that a difference in K^+ activation of the Na^+ pump accounts for the difference in rat heart muscle in its response to K^+.

In rat heart muscle preparations stimulated at 1.5 Hz, K^+ produces biphasic effects on the force of contraction. When K^+ was elevated from 1 mM to 3 mM, developed tension decreased. Further increase in K^+ up to 9 mM caused a marked increase in developed tension. Several lines of evidence suggest that $[Na^+]_i$ probably plays an important role in the K^+-induced biphasic inotropic effect. It can be argued that K^+ may have two independent effects: one is the stimulation of the Na pump which reduces the amount of Ca^{2+} available for contractile activation (Eisner and Lederer, 1980b, Im and Lee, 1984) and the other is to increase a specific pool of Ca^{2+}which contributes to the manifestation of the PIE.

Under conditions in which $[Na^+]_i$ is already high, K^+ would have a greater stimulatory effect on the Na pump. In contrast, the Na pump cannot be stimulated when $[Na^+]_i$ is low and therefore is the rate-limiting step of the Na pump turnover. Under the latter condition the K^+-induced PIE should be more prominent. These explanations appear to be consistent with the present results.

The observation that K^+ produced a greater positive inotropic effect when the frequency of stimulation was lower supports the above hypothesis. Although $[Na^+]_i$ was not assayed in the present study, it has been well established that $[Na^+]_i$ is influenced by the frequency of stimulation (Langer, 1974). Cohen et al (1982) have shown a good correlation between stimulation frequency and $[Na^+]_i$ activity in guinea-pig and sheep ventricular muscle and in sheep Purkinje fibers strands. In the above preparations, rate- and time-dependent increases in $[Na^+]_i$ activity were detected at a stimulation frequency as slow as 0.2 Hz. However, whether the stimulation frequency influences the $[Na^+]i$ in the rat heart is controversial. In order to explain the unique characteristic of the rat heart, namely a decrease in the developed tension associated with an increase in frequency of stimulation, it has been suggested that "Na pump lag" may be absent in the rat myocardium (Blesa et al., 1970; Langer, 1975b). Presently, there are no reports on direct measurement of $[Na^+]_i$ activity in the rat heart to support or refute this argument.

The results of the present study indicate that the positive force-frequency relationship can be observed when atrial muscle preparations were treated with ryanodine. Even though the exact mechanismof action of ryanodine is still not totally understood (as will

be discussed later), it is generally agreed that ryanodine inhibits the intracellular Ca^{2+} pool which contributes to contractile activation especially when cardiac muscle is stimulated at low frequency. When the ryanodine-sensitive Ca^{2+} pool was eliminated after a 90 min incubation in the presence of 6 nM ryanodine, increase in the frequency of stimulation enhanced developed tension. It may not be unreasonable to assume that an increase in $[Ca^{2+}]_i$ is secondary to an elevation of $[Na^+]_i$. Preliminary results indicate that $[Na^+]_i$ was indeed elevated in rat heart muscle at high frequency of stimulation (unpublished data). Therefore, in the present study, it is likely that the $[Na^+]_i$ in the rat heart muscle stimulated at 1.5 Hz was higher than that under 0.5 Hz stimulation. Addition of K^+ causes a greater stimulatory effect on the Na pump under 1.5 Hz than under 0.5 Hz stimulation. The end result would be a greater K^+-induced PIE when the stimulation frequency is lower because the PIE would not be masked by the negative inotropic effect which is caused by Na pump stimulation. Such results were observed in the present study. When the frequency of stimulation was further increased to 3 Hz, the onset of the PIE by K^+ was delayed and the PIE of K^+ was smaller than at the lower frequency of stimulation. This is indicative of an enhanced Na pump stimulatory effect by K^+ when $[Na^+]_i$ is elevated by the higher stimulation frequency. This effect of K^+ would thus mask the PIE of K^+. Once the $[Na^+]_i$ becomes a limiting factor and Na pump cannot be further stimulated, the PIE of K^+ is then manifested.

Veratridine is an agent which prolongs the open state of the Na^+channel (Sperelakis and Pappano, 1969; Komey et al., 1980). Veratridine(1 μM) caused a larger PIE which is probably due to an

increase in $[Na^+]_i$ and secondarily to an increase in $[Ca^{2+}]_i$ due to modification of Na^+-Ca^{2+} exchange. Under these conditions, an elevation of K^+ concentration up to 9.5 mM could no longer induce a PIE. Lowering the incubation temperature can also elevate $[Na^+]_i$ by reducing the turnover rate of the Na pump. Under these conditions, the K^+-induced PIE was attenuated. At 26oC, the PIE of K^+ at 0.5 Hz was smaller than that at 30oC. At 1.5 Hz, K^+ only induced a transient PIE, probably due to further accumulation of $[Na^+]_i$ at the higher frequency of stimulation. These findings are consistent with the hypothesis that when $[Na^+]_i$ is not limiting, the major effect of K^+ is to stimulate the Na pump which would result in a negative inotropic effect, and this effect would counter-balance the inherent K^+-induced PIE observed in the rat heart.

The observation that under low stimulation frequency, the K^+-induced PIE can be observed in atrial muscle preparations of guinea-pig provides additional support for the above hypothesis. It can be argued that $[Na^+]_i$ is lowered by the extremely low rate of stimulation (0.25 Hz). Under these conditions, the stimulatory effect of Na pump by K^+ is likely to be limited. The PIE of K^+ on the guinea-pig heart, which is usually masked by the Na pump stimulatory effect of K^+ when stimulation frequency is high, could then be expressed.

An alternative explanation may be possible for the observation that the PIE of K^+ is greater when the frequency of stimulation is low. When cardiac muscle preparations were stimulated at low frequencies, the relative importance of the surface Ca^{2+} pool decreases whereas that of

the Ca^{2+} pool in the sarcoplasmic reticulum increases. Therefore, it may be argued that the PIE of K^+ is more prominent when the Ca^{2+} pool in the sarcoplasmic reticulum plays a major role in contractile activation. This explanation, however, is not tenable because ryanodine, which eliminates the contribution of the intracellular Ca^{2+} pool, failed to attenuate the K^+-induced PIE.

The observation that rat atrial muscle preparations remained stable at $[K^+]_o$ as low as 1 mM while guinea-pig muscle preparation displayed arrhythmias when $[K^+]_o$ was as high as 3.5 mM merits some discussion. A higher $[Na^+]_i$ in guinea-pig myocardium could explain such a difference. It could be speculated that the Na pump in guinea-pig cells usually operates with a lower reserve capacity (Akera et al., 1979). A slight inhibition of the Na pump could lead to Na^+ accumulation and Ca^{2+} overload via Na^+-Ca^{2+} exchange and then arrhythmias occur. The smaller reserve capacity could be due to a greater Na^+ influx rate and/or a smaller number of Na pump sites. Na^+ influx is believed to be highest during phase 2 of the action potential (Langer, 1974). The guinea-pig heart has a large plateau phase and thus may be expected to have higher $[Na^+]_i$. However, there is no existing data to support or disprove this point. Because there is no drastic differences in the number of Na pump site in cardiac cells of guinea pig and rat (preliminary data from our laboratory), Na pump densities are unlikely to contribute to the differences in stability of the rat and guinea-pig cardiac tissue under low $[K^+]_o$ incubation.

Differences in resting membrane permeability to Na^+ and K^+ between rat and guinea-pig atria could also be a possible explanation for the instability of guinea-pig atria in low $[K^+]_o$. For instance, the Na^+

permeability of the guinea-pig atria may be greater than that of rat atria. If this was the case, not only the $[Na^+]_i$ could be higher, but also the RMP of the guinea-pig atria would be expected to be less negative than the rat atria at the same $[K^+]_o$ according to the Goldman equation. Reducing the $[K^+]_o$ would then cause the RMP of the guinea-pig atria to depolarize to a state where extrasystoles could occur. However, preliminary results indicate that the Na^+ permeability in the puinea-pig atria is probably not substantially different from the corresponding value in the rat atria. First, the RMP of guinea-pig atria at 4.0 mM $[K^+]_o$ was -75.6 ± 1.4 mV (n=6, data not shown) which is just slightly less negative than the RMP of rat atria at 3.5 mM $[K^+]_o$ (-78.5 ± 1.4 mV, n=8). The difference is attributable to a slight difference in $[K^+]_o$. Second, changing the $[K^+]_o$ from 4.0 mM to 9 mM caused the RMP to depolarize to -58.9 ± 3.9 mV (n=3, data not shown) which is similar in magnitude as that observed in the rat atria (-59.0 ± 1.2 mV at 9 mM $[K^+]_o$, n=8). Therefore, there is no indication that Na^+ and/or K^+ permeability of guinea-pig atria is different from that in rat atria.

These data would seem also to argue against that differences in resting Na^+ and K^+ permeability as a possible explanation for the different inotropic response of rat and guinea-pig atrial preparations to changes in K^+ concentrations.

In conclusion then, depletion of $[K^+]_o$ does lead to accumulation of $[Na^+]_i$ in the rat heart as in other species as indicated by the increase in developed tension. Elevation of $[K^+]_o$ lead to stimulation of the Na pump and a negative inotropic effect; however, when $[K^+]_o$ is higher than 3-4 mM, the positive inotropic effect of K^+ prevails. Conditions which modify the $[Na^+]_i$ also modify the K^+-induced PIE. When $[K^+]_i$ was

elevated by increasing frequency of stimulation, addition of veratridine or lowering incubation temperature, the K^+-induced PIE was attenuated. When $[Na^+]_i$ was reduced by extremely low frequency of stimulation, the PIE of K^+ can also be expressed in guinea-pig heart muscle. Thus, intracellular [Na+] controls the expression of the K^+-induced PIE by regulating the effect of K^+ to stimulate the Na pump; when Na pump stimulation by K^+ is great, the PIE of K^+ is small.

B. Electrophysiological Properties of the Rat Heart and the Positive Inotropic Effect of K^+

The relationship between electrophysiological changes which occur in the cardiac muscle and the alterations of the force of contraction is undoubtly complex (Winegrad, 1979). Attempts to explain the PIE of K^+ from electrophysiological changes would seem to be even more difficult because K^+ has been shown to change more than one electrical property of the cardiac cell (Noble, 1976; Morad, 1980). In the present study, attempts were made to detect any electrophysiological changes that are uncommon to any known effect of K^+ observed in cardiac tissue of other species. Such an abnormality could be a possible explanation of the paradoxical inotropic effect of K^+ on the rat heart. Although the PIE of K^+ could also be shown in the guinea-pig heart muscle, the K^+-induced PIE was much more prominent in the rat heart. Such a difference may suggest a possible unique feature in the rat heart that could be easily studied.

In the present study, elevation of $[K^+]_o$ caused a significant prolongation of the APD of the rat heart. This effect of K^+ is different from that of K^+ observed in other species. K^+ has been shown

to shorten the APD in most species studied (Weidmann, 1956; Deleze, 1959, Vassalle, 1966). The reason for such a shortening of the APD was proposed to be due to an increase in the time-independent outward current (ik_1) with elevated $[K^+]_o$ (Noble, 1976; Cleeman,1981). However, in the rat heart, K^+, although depolarized the RMP, does not seem to significantly affect the cross over of the inward rectifying background current between -60 to -30 mV. This probably explains the failure of K^+ to shorten APD in the rat heart.

Among the electrical properties, the changes in APD have been correlated with changes in the force of contraction. Wood et al. (1969) showed that in calf or sheep ventricular fibers a constant depolarizing current prolonged the APD and at the same time increased the force of contraction. Conversely, a hyperpolarizing current shortened the APD and reduced the force of contraction. Both the positive and negative inotropic effect required six to eight beats to reach steady state. These investigators suggested that the concentration of intracellular Ca^{2+} at each beat is partly determined by the duration and magnitude of the plateau phase of the prior action potential. Morad and Trautwein (1968; 1973) conducted similar studies and came to the same conclusion.

In the rat heart, elevation of $[K^+]_o$ at 1.5 Hz caused a significant prolongation of T_{20} and T_{50} (Table 1). The effect of K^+ on the T_{90} is more complex because K^+ caused a transient shortening of the T_{90} (shortened significantly 1 min and 5 min after elevation of $[K^+]_o$ but no significant change 10 min after elevation of $[K^+]_o$). The prolongation of T_{20} and T_{50} seems to be consistent with the hypothesis that inactivation of the I_{EO} by the depolarizing effect of K^+ prolongs the APD of the rat heart. The mechanism for the shortening of T_{90} is less

clear. Activation of a Ca^{2+}-activated outward K^+ current as reported in the Purkinje fibers (Siegelbaum and Tsien, 1980) could be a potential explanation for the APD shortening effect of K^+ (It should be noted that K^+ produced PIE in the rat heart which could suggest an elevation of $[Ca^{2+}]_i$). However, present data do not reveal the existance of such a current. As pointed out earlier, elevation of $[K^+]_o$ did not cause a shift of the inward-rectifying current as reported in other species and thus cannot be the reason for the shortening of T_{90} by K^+. Furthermore, elevation of $[K^+]_o$ caused a significant prolongation of the T90 when the temperature was lowered to 26oC (Table 2). The significance of the shortening of T_{90} at 30oC by K^+ is thus not clear.

Although the paradoxical APD lengthening effect of K^+ seems to be an attractive explanation for the K^+-induced PIE, the significance of this "paradoxical" effect is not clear. If APD is the only factor in controlling the K^+-induced PIE, then interventions that alter the K^+-induced PIE should be accompanied by a corresponding change in APD. At 26oC an elevation of K^+ from 3.5 to 9.5 mM caused a 24% increase in APD when rat atrial muscle preparations were stimulated at 1.5 Hz. Elevation of K^+ caused only an 8% increase in APD when stimulation rate was at 0.5 Hz. Nevertheless, K^+ caused significantly greater PIE at 0.5 Hz than at 1.5 Hz. These results suggest that APD is not the only factor in determining the PIE of K^+. However, these experiments do not preclude the possibility that prolongation of APD contribute partly to the K^+-induced PIE.

In the same series of experiments, increasing the stimulation frequency from 0.5 Hz to 1.5 Hz caused no change in APD of the rat atrial muscle when $[K^+]_o$ was at 3.5 mM. However, the APD (T_{20} and T_{50})

was significantly prolonged by increasing the frequency to 1.5 Hz when $[K^+]_o$ when $[K^+]_o$ was at 9.5 mM. This interval-duration relationship observed in rat atrial muscle is somewhat different from most other species in which an increase in stimulation frequency usually causes a shortening of the APD (Boyett and Jewell, 1980). Data on the interval-duration relationship of rat atria are not available. However, there are a few reports concerning the interval-duration relationship of the rat ventricular muscle. Nevertheless, these data are rather inconsistant ranging from no change (Blesa et al., 1970) to shortening (Payet et al., 1981), to lengthening (Lazarus et al., 1980) of the APD when stimulation frequency was increased. Watanabe et al. (1983) reported a lengthening of T_{25} and a shortening of T75 as the stimulation frequency was increased from 0.1 Hz to 5 Hz. Therefore, it seems difficult to compare the present result with the existing reports.

The reason for the prolongation of T_{20} and T50 in the present study is not clear. Watanabe et al. (1983) spectulated that the prolongation may be due to the incomplete recovery of a transient outward current when preparations were stimulated at high frequency as in the case of cardiac Purkinje fibers (Fozzard and Hiraoka, 1973; Hiraoka and Hiraoka, 1975). However, such an explanation does not seem to fit the present data because 1). the transient outward current is likely to be inactivated at 9.5 mM K^+ and 2). as shown by Josephson et al. (1984) the transient outward current in the rat myocytes had a very fast recovery time (time constant was 25.3 ms).

The effect of K^+ on the APD reported here, however, is quite different from that reported by Michell et al. (1984) who reported a shortening of APD when $[K^+]_o$ was increased from 1.2 mM to 3.8 mM and

from 3.8 mM to 12 mM (by comparing action potential configurations). It was speculated, without voltage clamp data, that K^+probably increase the inward-rectifying outward current similar to that observed in heart muscle of other species. However, as discussed earlier, K^+ does not seem to affect the cross over of the inward rectifying background current between -60 to -30 mV. The reason for the difference between their results and ours is not clear. However, their action potential records indicate that there is no change in RMP when $[K^+]_o$ was altered. No explanation is given for the failure of $[K^+]_o$ in the range of 1.2-12 mM to alter RMP.

In order to examine the currents underlying the action potential of the rat cardiac cells, enzymatically dispersed cells were used in the present experiments. The isolated cell seems to have electrophysiological parameters very similar to that of multicellular preparations, i.e. APD and RMP (Table 2 and table 3). As indicated by Pelzer et al. (1984) RMP close to multicellular preparations (-70 to -80 mV) could be recorded from isolated myocytes by the use of suction pipettes; Powell et al. (1979) recorded RMP less negative than -40 mV from isolated rat heart cells in KH buffer containing 3.8 mM K^+ using the conventional glass electrodes.

Although transient or early outward currents have been reported in nerves and cardiac Purkinje fibers, the existence of the I_{EO} in rat heart muscle cells seems to be unique. Very recently, Josephson et al. (1984) reported the presence of an early outward current in isolated rat ventricular myocytes. The current (I_{EO}) has almost identical characteristics as the one reported here. The above investigators demonstrated that the I_{EO} was not Ca^{2+}activated, was sensitive to

4-aminopyridine and inactivated at very negative potentials (-50 mV to -40 mV). The I_{EO} in the rat myocytes thus seem to be inactivated at a more negative potential than those from Purkinje fibers (approximately -20 mV, Siegelbaum and Tsien, 1980). They also reported that $Ba^{2+}$and Cs^{+}, which blocks K^{+}current, blocked the I_{EO}, suggesting that the current is conducted through K^{+} permeable channels.

The physiological significance of this transient outward current in the cardiac muscle cell is not clear. In Purkinje fibers, the Ca^{2+}-activated outward current has been shown to contribute to the rapid repolarization of phase 1 which is a characteristic of the action potential of Purkinje fibres. Siegelbaum and Tsien (1980) suggested that this Ca^{2+}-activated transient outward current might play a major role in offsetting the charge carried by the slow inward current. A Ca^{2+}-activated channel seemed to offer a convenient mechanism for automatically adjusting the outward current as the slow inward current is modulated. The I_{EO} in the rat cardiac cell, however, is not Ca^{2+}-activated. Therefore, it seems unlikely that this outward current would have a similar function as that proposed for Purkinje fibers. A transient outward current has also been reported recently in myocytes from rabbit subsidiary pacemaker tissue (van Ginneken and Giles, personal communication). It was suggested that such a current might modulate the frequency of firing by changes in effective or relative refractory period as a consequence of attenuations in the APD.

The I_{EO} is most likely responsible for the very short APD of the rat heart. Such a short APD would greatly limit the influx of Ca^{2+} during membrane depolarization. In view of the large intracellular Ca^{2+} store in the rat heart, the limited Ca^{2+} influx is likely to play the

role of trigger-Ca^{2+} if it has a significant role in contractile activation.

The possibility that the I_{EO} might be related to the K^+-induced PIE in the rat heart was examined. Voltage clamp experiments demonstrated that the I_{EO} was totally inactivated at a membrane potential of -60 mV. Because elevation of $[K^+]_o$ from 3.5 mM to 9.5 mM depolarized the RMP about 20 mV, it is possible that the I_{EO} is inactivated in the presence of elevated $[K^+]_o$. Inactivation of an outward current could lead to prolongation of the APD and thus increase Ca^{2+} influx. In the present study, 4-aminopyridine (3 mM) was shown to be capable of eliminating the I_{EO} (from voltage clamp experiments) and producing a large PIE in the rat heart. However, in the presence of 3 mM 4-aminopyridine, K^+ still produced a large PIE in the rat heart suggesting that elimination of I_{EO} is not the only mechanism of the PIE of K^+ although it may contribute to the K^+-induced PIE if and when it occurs. The concentration of 4-aminopyridine used should cause a sufficient suppression of most, if not all, of the I_{EO} in the present study. First, from the voltage clamp experiment, 3 mM 4-aminopyridine seems to be able to totally eliminate the I_{EO}. Second, addition of 4-aminopyridine higher than 3 mM caused only a slight further increase in developed tension. Furthermore, the fact that K^+ also produced a PIE in left atrial muscle of guinea-pig heart when the preparation was stimulated at a very low frequency strongly suggests that I_{EO} cannot be the only contributor to the K^+-induced PIE. A transient outward current has not been demonstrated in guinea-pig cardiac muscle cells (Hume and Uehara, personal communication)

C. The Ca^{2+} Pool Involved in the K^+-induced PIE

In cardiac tissue, two major sources of Ca^{2+} are involved in the activation of the contractile process. That in the extracellular space including the surface of the sarcolemma and the intracellular Ca^{2+} pool located in sarcoplasmic reticulum. The relative contribution of Ca^{2+} from these two sources vary depending upon species and tissues (Fabiato and Fabiato, 1977; Bers et al., 1981).

1. The Extracellular Ca^{2+} Pool

Unlike skeletal muscle, the contractility of mammalian myocardium is extremely sensitive to the Ca^{2+} concentration of the extracellular fluid. The removal of $[Ca^{2+}]_o$ results in a rapid decline in the force of contraction (Ringer, 1883). Although $[Ca^{2+}]_o$ undoubtedly plays a regulatory role in myocardial contraction, the quantity of Ca^{2+} from this source which is actually delivered to the myofibrils is uncertain (Langer, 1984) especially in rat heart muscle. Recent evidence indicates that the regulatory extracellular Ca^{2+} is bound to the sarcolemmal membrane. Langer et al. (1975b) emphasized the importance of the sialic acid-bound Ca^{2+} in the glycocalyx. A more recent paper, however, puts emphasis on the importance of the phospholipid bound Ca^{2+} of the sarcolemmal membrane (Philipson, 1980a). Displacement of the sarcolemmal Ca^{2+} pool by ions such as La^{3+} which cannot enter the cell caused a rapid decrease in contractile force (Sanborn and Langer, 1970; Philipson and Langer, 1979). Bers and Langer (1979) showed that the order of affinity of divalent and trivalent cations for sarcolemmal Ca^{2+} binding sites was the same as the order of potency of these cations to cause excitation-contraction uncoupling observed in neonatal rat papillary muscles ($Cd^{2+} > Co^{2+} > Mg^{2+}$; $Y^{3+} >$

$Nd^{3+} > La^{3+}$). Ber et al (1981) later demonstrated that cardiac tissues which have less well- developed SR such as the neonatal rat, rabbit and frog ventricles have a close correlation between sarcolemmal Ca^{2+} binding and contractility. Those tissues which have a well-developed SR system such as adult rat ventricle and rabbit atrium lack such a correlation. These authors suggested that the Ca^{2+} bound at sites on the sarcolemmal membrane was adequate to support tension development in the neonatal rat, rabbit and frog ventricular muscle. Thus, in species with a less developed SR, the extracellular Ca^{2+} pool appears to play a role in activating Ca^{2+}. It should be noted that the elimination of Ca^{2+} from the incubation medium also causes a rapid loss of contraction in species with a well-developed SR. Therefore, the sarcolemmal membrane-bound Ca^{2+} probably functions as a trigger Ca^{2+}, rather than in direct regulation of cardiac contractility, in the rabbit atrial or adult rat ventricular muscle.

In the present study, Cd^{2+} was used to limit the contribution of this superficial Ca^{2+} pool. Cd^{2+} was chosen because it does not precipitate in the phosphate-containing buffer at the highest concentration used. The effectiveness of Cd^{2+} is demonstrated by the large decrease in developed tension after its addition, probably owing to displacement of sarcolemmal-bound Ca^{2+} and a subsequent reduction of Ca^{2+} influx. Voltage clamp experiments indeed indicated that such a concentration of Cd^{2+} (10 μM) was effective in suppressing the slow inward current (data not shown), as have reported by other investigators (Adams, 1980; Hume and Giles, 1983). Depletion of this superficial Ca^{2+}pool seems to attenuate the K^{+}-induced PIE. Developed tension was reduced drastically in the presence of 10 and 20 μM Cd^{2+}. Bers and

Langer (1979) have shown that Cd^{2+} possesses a high affinity for Ca^{2+} binding sites. Addition of K^+ (6 mM) to muscle preparations that were incubated with 10 uM Cd^{2+} for 90 mins induced a small sustained PIE. Although the percentage increase was comparable to that of control in the absence of Cd^{2+}, the actual increase in gram tension caused by K^+ was quite small. It suggests that Cd^{2+} may have depleted the Ca^{2+} pool which contributed to the K^+-induced PIE. At a higher concentration of Cd^{2+} (20 μM) K^+ could no longer produce a sustain PIE, and only, a transient PIE was observed. Cadium ion thus seem to inhibit the K^+-induced PIE in a dose-dependent manner. The decline in developed tension after the initial increase may be indicative of a stimulatory effect of K^+ on the Na pump. These results suggest an involvement of the superficial Ca^{2+} pool in the K^+-induced PIE.

2. The Intracellular Ca^{2+} Pool

The intracellular Ca^{2+} pool is another major source of Ca^{2+} for the contractile process. Specifically, the sarcoplasmic reticulum is believed to be the major Ca^{2+} storage site. Fabiato and Fabiato (1977) have proposed a model in which a small amount of Ca^{2+}, which enters the cardiac cell during membrane excitation, causes a graded release of Ca^{2+} from the SR.

In the present study, caffeine and ryanodine were used to study the contribution of intracellular Ca^{2+} to the K^+-induced PIE. Caffeine has been shown to inhibit Ca^{2+} transport into the SR in skeletal muscle (Endo, 1975a, b), in intact cardiac tissue (Henderson et al., 1974; Chapman and Leoty, 1976; Blinks et al., 1972) and in skinned cardiac cells (Fabiato and Fabiato, 1975). Using isolated SR preparations, Blayney et al. (1978) showed that caffeine in 1-10 mM

concentrations impairs active Ca^{2+} accumulation by making SR vesicle membranes more permeable to Ca^{2+}. Hunter et al. (1982) have been able to demonstrate, by using a $^{45}Ca^{2+}$ wash-out technique, a Ca^{2+} pool which was rapidly released upon addition of caffeine (10 mM) and was sensitive to $[Ca^{2+}]_o$ and protected by procaine. There is general agreement that the primary mechanism of action of caffeine at this high concentration is depleting the Ca^{2+} store at the S.R.

In the present study, caffeine (3 mM) totally abolished the post-rest potentiated contraction with little effect on developed tension observed under 0.5 Hz. This latter effect of caffeine on the rat heart is somehow different from that reported in other species in which a larger positive inotropic effect could be observed (Blinks et al., 1972). This PIE is most likely due to the phosphodiesterase inhibitory effect of caffeine (Blinks et al., 1972; Henderson, et al; 1974). As pointed out by Henderson etal. (1974), the effect of caffeine on developed tension was species- dependent. Caffeine caused a positive or negative inotropic effect on cat myocardium depending on stimulation frequency, however; caffeine caused a negative inotropic effect on rat myocardium under all conditions. This species difference is probably related to the relative contribution of intracellular Ca^{2+} pool to the contractile process. The difference between the present data and that of Henderson on the response of rat cardiac tissue to caffeine is not clear. It could be due to differences in temperature, $[K^+]_o$, buffers and/or frequency of stimulation which may affect the relative contribution of extracellular and intracellular Ca^{2+} pools to the process of contraction.

In the present study, 3 mM caffeine seems to be effective in

depleting the SR Ca^{2+} pool judging from the disappearance of the post-rest potentiated contraction. Under these conditions, the K^+-induced PIE was also totally abolished. This result seems to suggest a possible involvement of an intracellular Ca^{2+} pool, either directly or indirectly, in the K^+-induced PIE.

Even though a recent report by Konishi et al. (1984) suggests that caffeine may directly reduce the Ca^{2+} sensitivity of the contractile system of the rat heart, these results are quite different from others which suggest an enhanced Ca^{2+} sensitivity of the contractile system by caffeine (McClellan and Winegrad, 1978; Mope et al., 1980; Wendt and Stephenson, 1983). Furthermore, a slight reduction of the Ca^{2+} sensitivity of the contractile system is unlikely to abolish the large K^+-induced PIE. Therefore, in the present study, it is assumed that caffeine abolishes the K^+-induced PIE by diminishing the Ca^{2+} pool which mediates the PIE of K^+.

Caffeine, at higher concentrations (10 mM), has also been shown to reduce Ca^{2+} current of sheep Purkinje fibers and cat papillary muscle in voltage clamp experiments (Eisner et al., 1974; Kohlardt et al., 1974). Accumulation of cytosolic free Ca^{2+} or the depletion of SR Ca^{2+} rather than Ca^{2+} channel blockade by caffeine per se had been postulated to be the primary cause of such an affect. Whether Ca^{2+} current blockade by caffeine plays a role in the present experiments is not clear. However, because developed tension of the rat atrial preparations after caffeine treatment (3 mM) was not attenuated, it seems doubtful that such a mechanism plays a significant role.

On the other hand, ryanodine did not seem to attenuate the K^+-induced PIE. Ryanodine has been shown to affect the function of

intact cardiac tissue (Hajdu and Leonard, 1961; Frank and Slector, 1975; Sutko et al., 1979; Sutko and Willerson, 1980). Evidence suggest that ryanodine may specifically alter the function of the SR. In isolated cardiac sarcoplasmic reticulum vesicles, ryanodine (in the millimolar range) enhanced $^{45}Ca^{2+}$ uptake (Jones et al., 1978). Because there was no increase in the $^{45}Ca^{2+}$ influx rate, the data suggest that ryanodine was capable of inhibiting Ca^{2+} release from the SR. Sutko and Willerson (1980) have later shown that the sensitivity of cardiac tissue to ryanodine was species dependent. Species, such as rat and dog, which depended more on intracellular Ca^{2+} (SR) for the contractile process were more sensitive to ryanodine. Ryanodine also inhibited post-rest contraction presumably by depressing the SR Ca^{2+} pool. The common conclusion from these experiments is that ryanodine exerts its effect by inhibiting Ca^{2+} release from the SR.

In the present study, ryanodine (6 nM) treatment depressed contractile force and eliminated the post-rest potentiated contraction of the rat heart. Despite the indication of an altered SR function, however, ryanodine pretreatment failed to suppress the K^+-induced PIE. These data suggest that the ryanodine-sensitive Ca^{2+} pool is not involved in the K^+-induced PIE, and that the ryanodine-sensitive Ca^{2+} pool is different from the caffeine-sensitive Ca^{2+} pool which is involved in the PIE of K^+. The latter Ca^{2+} pool may or may not be related to S.R.

The inability of caffeine to abolish the K^+-induced PIE in the presence of ryanodine is rather intriguing. It suggests that although the caffeine-sensitive Ca^{2+} pool is involved in the PIE of K^+, it is probably not absolutely required for the expression of the K^+-induced

PIE. Furthermore, the data suggest that ryanodine in addition to diminishing an intracellular Ca^{2+} pool, may have created a "new" Ca^{2+} pool, responsive to activation by K^+. It should be noted that without ryanodine, K^+ could not produce a PIE in the presence of caffeine. The fact that in the presence of ryanodine, K^+ increased the steady-state developed tension without full recovery of the diminished post-rest potentiated contraction suggests that the Ca^{2+} pool being activated is probably different from the SR Ca^{2+} pool.

Data by other investigators seem to support an alternative mechanism of action of ryanodine. Ryanodine depressed cardiac function in the nanomolar range (Sutko and Willerson, 1980) but inhibition of $^{45}Ca^{2+}$ release from isolated SR did not occur until ryanodine concentration reached the millimolar range (Jones et al., 1978). Thus, the mechanism of action of ryanodine at the very low concentration seems unclear. These investigators also showed that ryanodine in millimolar concentrations greatly accelerated the rest-induced decay of developed force in rat ventricle. The rest-induced decay of contraction is believed to reflect the rate of loss of the SR Ca^{2+} during the quiescent period (Allen et al., 1976). On the other hand, ryanodine in micromolar concentrations has been shown to increase $^{45}Ca^{2+}$ release from guinea-pig atrial muscle preparations (Frank and Slector, 1975) and canine papillary muscle (Naylor et al., 1970). These finding are consistent with the well-known fact that ryanodine induces irreversible contracture in skeletal muscle (Jenden and Fairhurst, 1969). Ryanodine has been shown to accelerate the loss of cellular $^{45}Ca^{2+}$ from rat skeletal muscle. Results of these studies suggest that, rather than inhibiting SR Ca^{2+} release, ryanodine may actually increase the release of Ca^{2+}

from SR. Using a $^{45}Ca^{2+}$ wash-out technique, Hunter et al. (1983) indeed was able to show that ryanodine in micromolar concentrations decreased a Ca^{2+} and caffeine-sensitive Ca^{2+}pool, but at the same time increased a more readily releasible Ca^{2+} pool. These investigators, suggested a relocation of the SR Ca^{2+} pool due to increasing leakage of the Ca^{2+} pool. The exact location of this Ca^{2+} pool is presently unknown.

Sutko and Kenyon (1983) have shown that ryanodine at high concentrations (1 μM) blocked a 4-aminopyridine insensitive outward current in calf Purkinje fibers. However, it is doubtful that this effect of ryanodine plays any role in the interpretation of the present results because the concentration of ryanodine used in the present experiments was only 6 nanomolar.

In conclusion, the superficial Ca^{2+} pool seems to be required for K^+-induced PIE. Although the caffeine sensitive Ca^{2+} pool is involved in the PIE of K^+, it may not be absolutely required. The Ca^{2+} pool which is attenuated by ryanodine does not seem to be involved in the K^+-induced PIE. However, evidence suggests that a Ca^{2+} pool activated by ryanodine could contribute to the K^+-induced PIE. Such a pool may substitute for the caffeine -sensitive Ca^{2+} pool.

SUMMARY and CONCLUSION

Elevation of $[K^+]_o$ produced both a negative and a positive inotropic effect in the rat heart. The negative inotropic effect is most likely due to stimulation of the Na pump. Mechanism(s) other than or in addition to the elimination of the I_{EO} seems to be the primary cause of the K^+-induced PIE. Conditions that increased the relative Na load of the Na pump attenuated or abolished the PIE of K^+. When the Na load was minimized, K^+ also produced a PIE in the guinea-pig atrial preparations. A superficial Ca^{2+} pool which is sensitive to Cd^{2+} seems to be essential for the expression of the K^+-induced PIE. A caffeine but not ryanodine sensitive Ca^{2+} pool seems to be involved in the PIE of K^+.

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BIBLOGRAPHY

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