Positive and Negative
Inotropic Effects of Elevated
Extracellular Potassium Level on
Mammalian Ventricular Muscle

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ABSTRACT The effect of moderate elevation in extracellular potassium concentration (up to 12 mm) on contraction of cat ventricular muscle was examined. Isometric force development was recorded from eight excised trabeculae and from six coronary-perfused in situ papillary muscle preparations. Contraction in the steady state was variably affected, sometimes decreasing monotonically, sometimes remaining unchanged, with increasing potassium level. In 11 of these 14 preparations, the steady state was preceded by a transient period in which the contraction was augmented. In addition, eight excised trabeculae were used in an experimental arrangement designed to distinguish between inotropic effects caused by potassium-induced alterations in the action potential and other, more direct, effects of this ion on contraction. The negative inotropic effect is attributable to a potassium-induced reduction in the amplitude and/or duration of the action potential plateau. The positive inotropic effect was found in experimental arrangements where effects of the potassium-rich medium on action potential time-course were effectively "buffered." The positive inotropic effect thus depends on the presence of the elevated potassium concentration and can occur independently of effects on the action potential time-course.

INTRODUCTION

Increases in extracellular potassium concentration to levels still compatible with propagated excitation have been reported by some investigators to depress contraction of mammalian ventricular muscle (Garb, 1951; Engstfeld et al., 1961; Sarnoff et al., 1966), while others have found it to be unaltered (Green et al., 1952; Goodyer et al., 1964). It is conceivable that potassium exerts its effects through more than one mechanism, which may vary in rela-
tive magnitude and in time-course, and thus account for these apparently conflicting reports. The present study concerns observations on the effects of moderate elevations in extracellular potassium concentration on contraction of thin excised cat trabeculae and of a coronary-perfused in situ cat papillary muscle preparation. The findings, briefly, are that there are two opposite effects of increased potassium level: (a) a negative inotropic one, which is related to potassium-induced alterations in the time-course of the transmembrane action potential; (b) a positive inotropic one, which occurs independently of such alterations.

METHODS

Isometric contraction was recorded from excised cat trabeculae, 0.8 mm or less in diameter, by a technique described previously (Kavaler and Morad, 1966). This involved mounting one end between Lucite blocks, with minimal crush of the muscle, and attaching the free end to a force transducer by means of a nylon snare. Eight of the muscles studied were mounted in this “block and snare” and were driven, by 5-msec pulses, at a rate of 15/min. One muscle, similarly mounted (Fig. 3, left), was driven at a faster rate of 30/min. In addition, in order to detect transient changes, a technique was used in which isometric force development was monitored from a papillary muscle, while its coronary circulation was being perfused with Tyrode solution. This preparation is illustrated in Fig. 1. Langendorff perfusion of a cat heart was carried out through an aortic cannula supplying oxygenated Tyrode solution containing 4% dextran, to minimize tissue edema. Coronary flow by gravity was of the order of 25 ml/min at a pressure head of 100 mm Hg. Right ventriculotomy was carried out and a papillary muscle disconnected from its valve attachments. A close-fitting ring, made of a paper clip, was mounted rigidly on a Prior micromanipulator (Eric Sobotka Co., Inc., Farmingdale, N.Y.) and restrained the septal attachment of the muscle. The plane of the ring was adjusted to make uniform contact with the underlying septal endocardium. Contraction was monitored from the tendinous end of the muscle by means of an RCA 5734 mechanoelectric transducer tube. The muscle was electrically driven by 5-msec pulses applied between the restraining ring and a Teflon-coated wire inserted into the septum 1 or 2 mm away. The drive rate was that which could capture, ranging from 45 to 88/min. Six preparations of this Langendorff type were studied.

To distinguish between effects on contraction which arise from the depolarizing consequences of elevated potassium levels and other possible effects of this ion, an experimental technique was devised to minimize potassium-induced depolarization. Results from eight technically satisfactory experiments of this type are reported below. Contraction was recorded from a very short segment of a muscle. The short segment length was 0.2 mm in the instance shown in Fig. 2 and 0.4-0.7 mm in other experiments. These lengths reflect some stretch from slack length and are estimated to represent more than 3/4 of the optimal length for contraction. In this segment, which was between 3/10 of a length constant, the transmembrane potential was strongly influenced by electrotonic effects from the remainder of the muscle, which was 4 mm or more in length. A thin cat trabecula of 0.2 mm² cross-section, or less, was drawn through
a close-fitting hole in a rubber partition, which separated two independently perfused chambers, as shown in Fig. 2. The long extent of the muscle, lying behind the partition in a narrow channel, was perfused by inflowing Tyrode (LS TYR) at a flow rate of 10 ml/min. A Tyrode jet (5 ml/min, SS TYR) made contact with the short segment. Stimulating electrodes were situated on each side of the insulating rubber partition, under and close to the muscle, which was driven at a rate of 24/min by pulses 0.8 msec in duration.

The force developed between the muscle’s anchorage at the rubber partition and a nylon snare coupling close to its distal end was monitored by an RCA 5734 mechano-electric transducer tube. That the contractile record was generated by the short muscle segment was indicated by a visible shortening (up to 1%) of that segment with contraction, including a brisk movement of the rubber partition toward the transducer coupling. In addition, on two occasions when the short segment was not me-
chanically or electrically viable, transmitted force from a vigorously lashing long segment was less than 2 mg, or 5% of the short segment values reported below (as grams per square millimeter). Finally, raising the calcium level from 1.8 to 4.5 mM only in the Tyrode perfusing the short segment caused the same increase in recorded force development (67%) as did a similar increase in calcium in the Tyrode perfusing both segments (68%). Applying the calcium increase only to the long segment did not increase the recorded contraction, but, as described in the Results, caused a small decrease (9%). This result also indicates that, when different Tyrode solutions are applied to the two segments, extracellular diffusion gradients of large magnitude do not extend far into the short segment. What gradients are present, even for an ion to which contraction is highly sensitive, do not have large effects on the recorded contraction.

Transmembrane potential was recorded differentially from KCl-filled glass microelectrodes through operational amplifiers in voltage follower configuration. Impalements were carried out very close to the end of the short segment near the transducer coupling (i.e., at least 95% of the way from rubber partition to nylon snare). Force and transmembrane potential time-courses were displayed on a Tektronix type 565 oscilloscope (Tektronix, Inc., Beaverton, Ore.) as well as on a Brush Mark 220 Recorder (Brush Instruments Div., Clevite Corp., Cleveland, Ohio). In addition to these tracings, as indicated in Fig. 2, right, the action potential upstroke was displayed on a 10 msec sweep. Stimulus duration was set at 0.8 msec. Stimulus intensity in the upper record is at just threshold level. An 8 msec conduction time to the impalement site can be seen (from anodal break excitation in the long segment). At a slight increase in stimulus intensity conduction time becomes virtually zero, as shown in the lower record. All experiments of this sort reported below meet this requirement, and thus effects of potassium level on conduction are not a factor in causing the observed effects on contraction.

In order to evaluate roughly the rapidity with which the composition of the extracellular fluid could be altered in these preparations, the time to a new contractile steady state was measured following a "step" increase in Tyrode calcium concentration. This time was 25 sec in an unusually thin (0.2 mm) excised trabecula mounted in a "block and snare," where the inflow consisted of a jet of Tyrode solution playing directly on the muscle (Fig. 3, left). In a representative coronary-perfused papillary muscle (Fig. 3, right) this time was 23 sec. The small degree of mechanical alternans, almost invariably found in the in situ preparation, can be seen. Still higher calcium levels brought the steady-state contraction to even greater values in both cases. Since stored intracellular calcium may exert a significant influence on contractile behavior of mammalian ventricular muscle (Wood et al., 1969), the new steady state for extracellular composition probably occurs earlier in time than the one for contraction shown in Fig. 3.

All experiments, including those on the Langendorff-perfused preparations, were carried out at temperatures between 26° and 28°C. The Tyrode solution had the following millimolar composition: NaCl 137; KCl 2.7; NaHCO₃ 11.9; NaH₂PO₄ 0.32; MgCl₂ 0.5; CaCl₂ 1.8; dextrose 7.0. A gas mixture of 96% O₂, 4% CO₂ was bubbled through the solution. Increases in potassium content were produced by addition of solid KCl to portions of a large amount of control Tyrode made up for each
experiment. The results shown in Figs. 6–10 were obtained during single microelectrode impalements which remained stable and were free of evident movement artefacts (one exception: Fig. 10, 15th beat) throughout the entire response to change of perfusing solution. In most cases this included the subsequent return to the control. All other data from excised tissues (Figs. 3–5) were obtained from the “block and snare” preparation referred to above (Kavaler and Morad, 1966).

RESULTS

The Negative Inotropic Effect

Fig. 4 shows the negative inotropic effect of moderate elevations in extracellular potassium level for five experiments with excised tissues ("block and snare") (left) and for six coronary-perfused in situ cat papillary muscle preparations (right). In the steady-state values shown in all graphs, contraction is progressively depressed in most muscles, with increasing potassium level, over the range 1.25–12 mEq/liter. In two excised trabeculae, there was no appreciable change in contraction over this range of potassium concentrations, and one of the in situ papillary muscles is only slightly affected. This finding applies both to developed force (lower graphs) and to peak rate of force development (upper graphs).

The Positive Inotropic Effect

In 11 of a total of 14 muscles studied (seven of eight "block and snare" preparations, four of six Langendorff-perfused muscles), transient augmentation of contraction occurred on elevation of the potassium level; this was followed,
in the steady state, by an ultimate depression of contraction relative to the control. The upper portion of Fig. 5 shows an experiment on a thin trabecula. Within 20 sec after exposure to 10.8 mM potassium Tyrode, peak augmentation of contraction (by 16%) has occurred. The transmembrane resting potential and the duration of the action potential are, as expected, reduced in the potassium-rich medium. In the steady state, as shown on the extreme right, contraction is reduced (by 13%) relative to the control. The tracing on the lower left shows the same effect in a coronary-perfused papillary muscle preparation. Peak augmentation of contraction (a downward-going trace) occurs within 8 sec after exposure to the potassium-rich medium. On

The range of potassium concentrations which elicit the positive inotropic effect is considerably lower than the potassium levels reported to cause release of tissue catecholamines (Haeusler et al., 1968). In addition, the positive inotropic effect was not associated with a decrease in time-to-peak of contraction (e.g., Fig. 5: 510 msec, compared with 505 msec control), a common characteristic of the effect of norepinephrine (e.g., Kavaler and Morad, 1966). Finally, the positive inotropic effect was observed in the presence of 3 × 10^{-6} M propranolol, a dose which almost totally abolished (i.e., reduced to 7%) the increase in force development brought about by added norepinephrine. For these reasons, potassium-induced release of chemical mediators
from sympathetic nerve endings is an improbable cause of the positive inotropic effect.

The Negative Inotropic Effect and Potassium-Induced Alteration of the Action Potential

The result of raising the extracellular potassium level to 10.8 mM in an entire trabecula (i.e., for both the long and short muscle segments of the preparation described in Fig. 2) can be seen in Fig. 6. There is a progressive reduction in resting transmembrane potential amounting to a depolarization of 19 mv in the steady state. The plateau of the action potential is concomitantly reduced both in amplitude and in duration. Associated with this is a reduction in conduction, as developed force or as peak rate of force development. The action potential upstroke is markedly reduced in the depolarized tissue, but this introduces no important element of conduction delay.

As shown in Fig. 7, essentially the same sequence of events is brought about by raising the potassium to 10.8 mEq/liter only in the Tyrode perfusing the long muscle segment. The short segment was 0.4 mm in length. The depolariz-
ing effects of elevated potassium thus can bring about a reduction in contraction, associated with a lowering and shortening of the action potential plateau. Return to the control state is shown for another preparation (short segment 0.7 mm) in Fig. 8. Voltage uniformity in this relatively long extent of muscle

![Diagram](image-url)

**Figure 6.** Time-course of electrical and mechanical changes in an excised trabecula (preparation shown in Fig. 2) on raising the potassium level in both short and long segments. In each set of traces the same, representative, control record is superimposed on one obtained at the indicated time (beat-to-beat interval of 2.5 sec) after raising the potassium concentration in the medium. The high potassium records show a progressive decrease in developed force ($P$), peak rate of force development ($\dot{P}$), and in amplitude and duration of the action potential plateau, as well as a progressive reduction in transmembrane resting potential ($\text{DEPOL}$). The fast sweeps display peak rate of rise of the action potential ($\dot{V}$) and the stimulus artefacts shown on these traces have a duration of 0.8 msec. Values for the control state are given in the column of figures at the extreme left.

![Diagram](image-url)

**Figure 7.** Negative inotropic effect of membrane electrical changes due to 10.8 mm potassium Tyrode, applied only to the long segment (preparation shown in Fig. 2). Symbols for measured variables are as in Fig. 6. Traces of a control beat are superimposed on the experimental traces, which show progressive reduction in transmembrane resting potential and in the amplitude and duration of the plateau.
is less than that shown in Fig. 7. Recovery of control values for contraction is associated with the reverse sequence of progressive increase in amplitude and duration of the action potential plateau which accompany reduction of the potassium level in the Tyrode perfusing the long segment, from 10.8 to 2.7 mM. This is the preparation whose response to elevation of extracellular calcium level is described in Methods. Raising the calcium only for the long segment did not increase contractile force of the short segment. The small decrease (9%) which occurred may be attributed to a 7 mv decrease in the amplitude of the action potential plateau in the calcium-rich medium. Thus, the reduced contraction shown in Fig. 8 is more reasonably attributed to an electro-

![Graph showing action potential plateau](image)

**Figure 8.** Restoration of contraction on returning the long segment to its control potassium environment. A representative trace during prolonged exposure of the long segment to 10.8 mM potassium Tyrode is superimposed on each record taken following return of normal 2.7 mM potassium Tyrode to the long segment (preparation as in Fig. 2, symbols as in Fig. 6). Control values for contraction, before application of the potassium-rich Tyrode, were $P$, 0.23 g/mm$^2$; $\dot{P}$, 1.22 g/mm$^2$-sec.

...tonically induced shortening of action potential duration than to an extracellular potassium diffusion gradient extending significantly into the short segment.

Elevation of the potassium level only in the short muscle segment was found to bring about a reduction in contraction in one preparation, of a total of eight, as shown in Fig. 9. Here, although alteration in resting potential was almost totally abolished, the plateau was markedly lowered within a few beats after exposure to 10.8 mEq/liter potassium. A possible explanation for such an alteration in plateau, when the resting potential is virtually unchanged, would be an increase in membrane potassium conductance, such as Carmeliet (1961) found in Purkinje fibers in the presence of an elevated extracellular potassium level. Also, in accord with this possibility is the fact that the resting membrane was actually **hyperpolarized** by 3-4 mv in this experiment, in the
first two beats following exposure to the 10.8 mEq/liter potassium Tyrode. Such an increase in membrane conductance for potassium might also account for the observed decrease in the rate of rise of the action potential upstroke.

*The Positive Inotropic Effect and its Independence of Changes in Action Potential*

In seven preparations contraction was increased on application of the potassium-rich Tyrode to only the short segment. In two of these, where the action potential was unaltered in the presence of the 10.8 mM potassium, the positive inotropic effect was maintained at a constant level throughout the period of exposure to the potassium-rich Tyrode. This is shown in Fig. 10. Although some depolarization of the resting level occurs, the plateau and repolarization time-courses are superimposable. A progressive increase in contraction is seen, which was maintained at the level shown in the 15th beat for a subsequent 40 sec period of exposure.

Fig. 11 shows the rapid onset and sustained character of the positive inotropic effect in another preparation where plateau and repolarization time-course were almost unaltered in the potassium-rich Tyrode. The 90% repolarization time, for example, was reduced by 11 msec from a control value of 465 msec. The overshoot and the early portion of the plateau were superimposable on the control record in the manner shown in Fig. 10. An "unbuffered" depolarization, equal in magnitude (8.4 mv) to that shown in Fig. 10, could be seen during the quiescent period between beats. The slight falloff from the peak value of contraction seen here was not observed in the experiment shown in Fig. 10, where action potential time-courses were exactly superimposable. In the other five preparations where potassium-rich medium, applied to the short segment, had a positive inotropic effect, more marked lowering of the action potential plateau and shortening of the repolarization...
time were seen. Falloff from a maximal positive inotropic effect was more marked in these trabeculae, resulting in two cases in a net reduction in contraction in the steady state. Reversal of the positive inotropic effect was regularly seen on return of the short segment to the 2.7 mm K+ Tyrode. In two preparations this was seen to occur in the absence of any change in the action potential time-course associated with the return of the extracellular potassium level to 2.7 mm.

**FIGURE 10.** Action potential-independent positive inotropic effect of potassium-rich medium applied to short (0.4 mm) segment. Preparation is as in Fig. 2, symbols are as in Fig. 6.

**FIGURE 11.** Contractile record (a downward-going trace) during positive inotropic effect of potassium-rich medium applied to the short (0.7 mm) segment. The highest value for developed force shown here (in the 10.8 mm K Tyrode) is 0.50 g/mm². Duration of exposure to the potassium-rich medium is exactly 1 min.

It is reasonable to assume that at the distal site on the short segment from which transmembrane potentials were consistently monitored, voltage control from the long segment would be least adequate. Thus, in the two short segments where plateau and repolarization time-courses in the presence of 10.8 mm potassium Tyrode were superimposable with those of the control, it is likely that the same was true at more proximal sites. In two preparations this was verified by a microelectrode impalement in the middle of the short segment during one application of 10.8 mm potassium Tyrode, and by an impalement at the usual distal site (at 0.95 of the short segment length) during
the next application of the potassium-rich medium. Depolarization of resting potential by the potassium-rich medium was less at the short segment's mid-point in both preparations, as was the degree of shortening of action potential duration. One of these experiments is shown in Fig. 12, where it can be seen that depolarization amounted to 10.5 mv at the distal site (0.38 mm of a 0.40 mm short segment) and 7.9 mv at the midpoint (0.18 mm from the rubber partition). The values for 90% repolarization time were 539 msec (at 0.38 mm) and 574 msec (at 0.18 mm).

![Figure 12](image-url)

**DISCUSSION**

Potassium-rich media can both augment and depress contraction of mammalian ventricular muscle. This finding provides an explanation for the apparently conflicting statements regarding potassium's effect. These effects would be expected to vary in their relative magnitudes in such a way as to cause, occasionally, no change in the steady-state contraction and this was the case for two of the excised trabeculae (Fig. 4, left), where the transient positive inotropic effect was particularly large (33 and 40% of control, respectively).

That the negative inotropic effect is associated with a reduction in the amplitude and/or duration of the action potential plateau is most convincingly shown by the experiments in which the potassium-rich medium was applied only to the long segment. These changes in plateau, imposed by the long segment, were uniformly associated with diminished contraction of the short segment. Elevation of extracellular calcium in the long segment failed to
increase short segment contraction. This indicates that major effects on contraction cannot reasonably be attributed to an extracellular diffusion gradient for potassium that extends into the short segment. Thus, potassium-induced changes in action potential time-course, and not the direct presence of the potassium-rich medium, appear to bring about the negative inotropic effect. Additional support for this view comes from the observation that, on application of the potassium-rich Tyrode to the short segment, an increasing negative inotropic component of the over-all effect on contraction was associated with reduction in plateau amplitude and/or duration. This ranged all the way from partial subsidence of the positive inotropic effect to a net reduction of contraction in the steady state. The mechanism by which an increase in extracellular potassium level affects the action potential plateau is not clear. For Purkinje fibers there is evidence that a reduction (depolarization) of the resting potential (van der Walt and Carmeliet, 1971) may contribute to this effect, as may an increase in membrane potassium conductance (Carmeliet, 1961). That membrane potassium conductance in ventricular muscle is also dependent, in this way, on extracellular potassium concentration is indicated by the constancy of intracellular potassium concentration over a wide range of extracellular concentrations (Page and Solomon, 1960) and by the observation that $^{42}\text{K}$ efflux increases with increasing extracellular potassium concentration (Weidmann, 1966). The contractile consequences of a reduction in plateau may plausibly be attributed to a decrease in a voltage-dependent calcium influx into the muscle fiber (Mascher and Peper, 1969; Beeler and Reuter, 1970 a).

Curtailment of the duration of depolarization can also be a factor in the reduction of force development by contracting ventricular muscle and such effects have been reported by Beeler and Reuter (1970 b). Whether this voltage-independent effect can be said to play a role in the negative inotropic effect would depend on one's estimate of the period of time during which force development is sensitive to duration of depolarization. The value of Beeler and Reuter (1970 b) of about 400 msec (for depolarization to about 0 mv) would appear to be applicable to these experiments, since it refers to effects on force development which are cumulative over several successive depolarizations, as are the effects reported here. The action potential curtailments shown in Fig. 7 are well within this 400 msec period, although the differing circumstances of experimental temperature (35°C) and of species (dog trabecula) in Beeler and Reuter's paper would preclude any final judgment on such a quantitative issue. Shorter periods of sensitivity of contraction to the duration of depolarization have been reported by Gibbons and Fozzard (1971) for Purkinje fibers of the sheep (100 msec for a depolarization to $-23$ mv, inside) and by Morad and Trautwein (1968) for cat trabeculae (200 msec of the naturally occurring action potential time-course). These estimates can be less easily related to the
results presented here, since they refer to effects on force development *during* the manipulation of *one voltage time-course* rather than to cumulative effects of repeated depolarizations, as is the case for the negative inotropic effect.

The positive inotropic effect appears to depend on the increased potassium concentration itself. It was observed in seven of eight preparations where the extracellular potassium level was raised in the short segment. In two preparations there was no alteration in the overshoot or early plateau of the recorded action potential. The remainder of the plateau and the repolarization limb were completely superimposable on the control in one and fell short of this by only 2% in the other. Microelectrode monitoring in both cases was from a distal point on the short segment where electrical “buffering” by the long segment should be least effective. This reasonable assumption was verified in two preparations. The positive inotropic effect has thus been seen in the total absence of alterations in the action (but not of the resting) potential.

The converse is also true: the positive inotropic effect was *never* seen in six preparations where the action potential was altered in the absence of an increase in potassium level (i.e., on raising the potassium level only in the Tyrode perfusing the long segment). Although it is possible that, in each case, a profound negative inotropic effect masked an underlying mechanism for augmenting contraction, this would be in great contrast with the 14 experiments (“block and snare,” Langendorff perfusion) in which the entire muscle was exposed to the potassium-rich Tyrode. In 11 of these a transient positive inotropic effect was seen. It is thus improbable that the positive inotropic effect is mediated by a potassium-induced reduction in the overshoot, or in the amplitude or duration of the action potential plateau. This finding is in no sense contradictory to that of Šumbera (1970) who showed effects of altering the overshoot and early plateau on the *same* beat, while the mechanical effects shown in these experiments are the cumulative results over an unknown number of beats (see Fig. 3, rapidity of exchange of extracellular fluid).

From the argument offered above regarding the total absence of the positive inotropic effect when the potassium level is raised only in the long segment, it may be regarded as unlikely that membrane depolarization during the resting state (i.e., diastole) is involved in the mechanism of the positive inotropic effect. This is in contrast to the effect of potassium-induced depolarization in prolonging the active state in skeletal muscle (Clinch, 1968; Chapman, 1969). Additional evidence on this point has been provided by Beeler and Reuter (1970b), who found that small depolarizations (e.g., 8 mv, as in Fig. 10 of the present study) have either no effect or a slightly depressant one on an immediately following contraction of a dog trabecula. Analogous contractile behavior of sheep Purkinje fibers has been reported by Gibbons and Fozzard (1971).
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