Inotropic Actions of Isoproterenol in Cat Ventricular Muscle

Effects of Extracellular Potassium

JAY R. WIGGINS

SUMMARY The inotropic actions of isoproterenol in cat papillary muscles or trabeculae bathed in a salt solution containing 4 mM KCl were compared to those in similar muscles bathed in a salt solution containing 22 mM KCl. Although isoproterenol evoked the same increase in force of contraction in both groups of muscles, the time course of contraction differed markedly. In muscles bathed in 4 mM KCl, isoproterenol caused a concentration-dependent decrease in time-to-peak force, but in muscles bathed in 22 mM KCl, isoproterenol caused a concentration-dependent increase in time-to-peak force. These data suggest that ventricular muscle activated by slow response action potentials may utilize a different mechanism of excitation-contraction coupling than do muscles activated by Na-dependent action potentials.


ISOPROTERENOL and other β-adrenergic agonists have positive inotropic effects in mammalian myocardium. In the presence of normal extracellular potassium (4 mM), the spectrum of this positive inotropic effect has been well established: isoproterenol increases both force of contraction and the maximum rate of force development while causing an abbreviation of both the time-to-peak force and total contraction time (Scholz, 1980). β-adrenergic agonists also enhance the slow inward current in potassium-depolarized cardiac fibers (for review, see Cranefield, 1975) and many investigators have made use of this property to induce “slow response” type action potentials in mammalian ventricular muscle. Watanabe and Besch (1974) showed that isoproterenol has a concentration-dependent positive inotropic effect in such “slow response-activated” muscle, and most investigators appear to have assumed that the inotropic actions of isoproterenol are identical in muscles activated by “fast response” and by “slow response” action potentials. However, there have been no systematic studies comparing this activity, and there are isolated observations (see for example, Fig. 4 in Becker et al., 1977) which suggest that, in slow response activated muscle, isoproterenol increases time-to-peak force. I have therefore, reexamined the inotropic actions of isoproterenol in cat ventricular muscle to assess the degree of which the inotropic activities of the drug are altered by extracellular potassium. The results show that the spectrum of inotropic activity of isoproterenol is markedly dependent on extracellular potassium; in the presence of elevated [K]o, the positive inotropic effect of isoproterenol is strongly dependent on an increase in time-to-peak force. One interpretation of these results is that the mechanism for excitation-contraction coupling changes in the presence of elevated [K]o. A brief report of these results has been published (Wiggins, 1980a).

Methods

Cats (1.5-3.0 kg, unselected for breed or sex) were obtained from the Hillsborough County (Florida) Animal Control Office and housed in the Vivarium for a minimum of 6 weeks to ensure that isoproterenol-responsiveness was normal (Wiggins, 1980b). After this conditioning period, the animal was anesthetized with pentobarbital Na (30 mg/kg, iv), and the heart was removed and rinsed in a physiological salt solution at room temperature. Papillary muscles or trabeculae carneae (>1 mm in largest diameter in situ) were ligated with 5-0 silk, tied to gold chain, and placed in a muscle bath for study under isometric conditions at 35° C. The muscle was stimulated through bare platinum wires (0.25 mm in diameter) placed parallel to the longitudinal axis of the muscle, using constant current pulses 5 msec in duration and approximately 10% above threshold. The average current strength was 5 mA. After stimulation was begun at a rate of 0.2 Hz, the muscle was gently and gradually stretched to the peak of its length-active force relation (i.e., at optimal length) and allowed to equilibrate for at least 45 minutes before the experiment was begun. If, at the end of this equilibration period, the muscle failed to develop at least 1 g/mm² of muscle cross-sectional area, it was discarded. When extracellular potassium was increased to 22 mM, muscles rapidly became inexcitable and failed to contract; for this...
series of experiments, then, stimulus strength was increased to 10 mA and pulse duration increased to 10 msec to restore contractile activity. Mechanical repriming was studied in some muscles by inserting an extrasyostole every 10 beats, followed by a compensatory pause, allowing post-extrasystolic potentiation to decay between test pulses. The test interval was increased in increments of 100 msec.

The output of the force transducer was amplified and electronically differentiated; both the primary and the differentiated signal were displayed on a rectilinear oscillographic recorder and read directly. Time-to-peak force was determined at a fast recording speed (125 mm/sec) by measuring the time from the onset of contraction until the first derivative of force (df/dt) crossed the zero-line. At the end of each experiment, the muscle was measured at 10x, cut between ties, and weighted. Muscle cross-sectional area was estimated by dividing the weight by the length, assuming a density of 1.0. Force and the first derivative of force were then normalized by dividing by cross-sectional area. Force and the maximum rate of force development (df/dt) were not further treated; the maximum rate of relaxation (−df/dt) was normalized by dividing by force to eliminate the load dependence of relaxation (Brutserst et al., 1978).

In some experiments, membrane potentials were measured, using glass microelectrodes filled with 3 M KCl (10-20 MΩ tip resistance). For these experiments, muscles were mounted as described above, except that approximately one-half of the preparation, distal to the transducer, was supported from below by a small acrylic pedestal to facilitate impalement by the microelectrode. The presence of the pedestal had little or no effect on force of contraction or the response to drug. The output of the microelectrode, suitably amplified, was displayed on an oscilloscope and photographed or recorded on the oscillographic recorder. For all studies on membrane potentials, multiple impalements (≥5) were made within a small (<0.25 mm²) area and the results averaged to form one datum point. Action potential variables measured were resting potential, overshoot of phase 0, plateau amplitude, action potential amplitude (the sum of the resting potential and either phase 0 or plateau amplitude, whichever was greater), the maximum rate of depolarization (dv/dtmax, obtained either by electronic differentiation with a differentiator linear between 10 and 1000 V/sec, or by measuring the slope of the action potential upstroke at a fast oscilloscope sweep speed), and action potential duration at 40 and 90% of repolarization (APD40 and APD90, respectively).

The salt solution contained (in mmol/liter): NaCl, 128; KCl, 4; CaCl₂, 2.5; MgCl₂, 0.5; NaHCO₃, 20; NaH₂PO₄, 1.8; dextrose, 5.5; disodium ethylene-diamine tetraacetic acid (Na₂EDTA), 0.05; and reduced glutathione, 0.0001. When KCl was increased to 22 mM, NaCl was decreased to maintain osmolality. Isoproterenol HCl (Sigma Chemical Co.) was dissolved in 0.1 mM reduced glutathione to retard oxidation and added directly to the perfusate. Concentration-response curves for isoproterenol were determined by cumulative addition of the drug. Phentolamine HCl (Regitine, a gift from Ciba-Geigy) was dissolved directly in the salt solution and allowed to act for at least 45 minutes before the experiment was begun. Phentolamine solutions were discarded after 2 hours.

Data were analyzed by t-test or by analysis of variance incorporating repeated measures (Myers, 1979). For ANOVA, subsequent comparisons were made using Scheffe’s method (Myers, 1979). A probability of 0.05 was considered significant for ANOVA or t-test; because Scheffe’s method is highly conservative, a probability of 0.10 was considered significant as suggested by Myers. Scheffe’s method was applied only when ANOVA showed significant differences in the data. Data were analyzed only for those muscles which met the minimum criterion for force development (see above) and in which isoproterenol caused no toxic effects (repetitive activity; spontaneous activity) within the concentration range of interest. Single aftercontractions were not considered to be toxic effects of the drug.

Results

Isoproterenol had a concentration-dependent positive inotropic effect both in muscles bathed in 4 mM KCl and in muscles bathed in 22 mM KCl. Results from typical experiments are shown in Figure 1; data from all experiments are summarized in Figure 2 and Table 1. In the presence of 4 mM KCl, isoproterenol increased both force and the maximum rate of force development. The threshold concentration for these effects was less than 3 nM (the lowest concentration studied), and increases in force were still seen with concentrations as high as 1 μM (data not shown). Most of these experiments were terminated after the addition of 100 nM isoproterenol because of the high incidence of toxic effects of the drug with higher concentrations. In contrast to the relatively simple changes in force and the maximum rate of force development, the changes in time-to-peak force induced by isoproterenol were biphasic. Isoproterenol 3 nM, increased time-to-peak force slightly (Table 2), whereas higher concentrations of the drug decreased time-to-peak force. The maximum rate of relaxation (−df/dt) increased with increasing concentrations of isoproterenol, but this increase was essentially dependent on the increase in force. The ratio of (−df/dt)/force was not changed significantly by isoproterenol in this study, although there was a high degree of variability in the data (Fig. 3).

Isoproterenol had a markedly different positive inotropic action in muscles bathed in 22 mM KCl. Again, there was a concentration-dependent increase in force and in the maximum rate of force...
development, the threshold concentration for which was less than 3 nM, and the increase in force evoked by the amine was approximately the same as that seen in muscles bathed in 4 mM KCl. However, the increase in df/dt was significantly less than that seen for comparable concentrations of isoproterenol in muscles bathed in 4 mM KCl, and time-to-peak force increased with increasing concentrations of the drug. Further, the maximum rate of relaxation increased to a much greater extent than did either the maximum rate of force development or force of contraction (Figs. 2 and 3). As can be seen in Figure 1, isoproterenol frequently caused the appearance of aftercontractions in muscles bathed in 22 mM KCl; these aftercontractions were also dependent on the concentration of isoproterenol in the perfusate. With the higher concentrations of isoproterenol, these aftercontractions often had faster rates of force development and relaxation than did the initiating contraction, even though force itself was less than that of the initiating contraction. These phenomena were not studied in detail, however. Qualitatively similar results were seen in muscles in which the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (10 and 100 µM) was used in place of isoproterenol (data not shown).

The time course of isoproterenol action also was affected by extracellular K. In muscles bathed in 4 mM [K]o, isoproterenol acted relatively quickly; a steady state was usually reached within 5 minutes after addition of the drug to the bath. In contrast, in muscles bathed in 22 mM [K]o, a steady state usually was not reached for 10–15 minutes after addition of the drug; there was, however, no difference in the latency of drug response when muscles were bathed in 22 mM [K]o.

In cat ventricular muscle, α-adrenergic agonists increase both force of contraction and time-to-peak force (Bruckner et al., 1978), suggesting that α-adrenergic receptors may be involved in the observed effect of isoproterenol to increase time-to-peak force in these experiments. To assess this possibility, muscles were exposed to 1 µM phentolamine for 45–60 minutes before isoproterenol was added to the perfusate. In the presence of 4 mM KCl, phentolamine blocked the increase in time-to-peak force evoked by 3 nM isoproterenol but did not alter the positive inotropic effect of the drug.
ISOPROTERENOL AND CONTRACTION IN HIGH [K] / Wiggins

721

\[ \frac{\Delta F}{\Delta t} (g/mm^2/s) \]

\[ -\frac{dF}{dt} (g/mm^2/s) \]

\[ TTP (msec) \]

**Figure 2** Inotropic actions of isoproterenol in cat ventricular muscle. The average effect of increasing concentrations of isoproterenol on force, maximum rate of force development (+dF/dt), maximum rate of relaxation (−dF/dt) and time-to-peak force (TTP) is shown for 10 muscles bathed in 4 mM KCl (O) and eight muscles bathed in 22 mM KCl (□). The maximum rate of relaxation is shown using filled symbols.

2. In contrast, for muscles bathed in 22 mM KCl, phentolamine (1 and 10 μM) did not alter the increase in force or time-to-peak force caused by any concentration of isoproterenol (data not shown).

**Table 2** Effect of 1 μM Phentolamine on the Response of Cat Ventricular Muscle to 3 nM Isoproterenol

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 11)</th>
<th>Phenolamine (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔF (g/mm²)</td>
<td>1.15 ± 0.33</td>
<td>1.97 ± 0.12</td>
</tr>
<tr>
<td>−dF/dt (g/mm² sec)</td>
<td>6.21 ± 2.09</td>
<td>6.36 ± 0.93</td>
</tr>
<tr>
<td>TTP (msec)</td>
<td>7 ± 1</td>
<td>1 ± 1*</td>
</tr>
</tbody>
</table>

Each value shows the mean ± SEM change evoked by isoproterenol. Muscles were exposed to phentolamine for 60 minutes before isoproterenol was added.

* P < 0.05.

The electrophysiological actions of isoproterenol in cat ventricular muscle are summarized in Table 3. In muscles bathed in 4 mM KCl, isoproterenol caused a concentration-dependent increase in plateau amplitude and action potential duration at both the 40 and 90% levels of repolarization. The increase in APD40 was more pronounced than the increase in APD90. In muscles bathed in 22 mM KCl, isoproterenol caused a concentration-dependent increase in overshoot, the maximum rate of depolarization, and APD90 (APD40 was not measured in these muscles).

The relationship between action potential duration and time-to-peak force is shown in Figure 4. For muscles bathed in 4 mM KCl, APD40 (corresponding to a membrane potential of approximately −20 mV) is plotted against time-to-peak force for increasing concentrations of isoproterenol. For muscles bathed in 22 mM KCl, APD90 (corresponding to a membrane potential of approximately −40 mV) is plotted against TTP. For muscles bathed in 4 mM KCl, there is a strong inverse correlation between time-to-peak force and action potential duration. In contrast, in muscles bathed in 22 mM KCl, there is marked positive correlation between action potential duration and time-to-peak force. This suggested that that positive inotropic action of isoproterenol seen under these conditions was linked to the prolongation of the action potential duration. However, since prolongation of action potential duration per se does not increase time-to-peak force in muscles bathed in normal K (see

**Table 1** Analysis of Variance for Mixed Design with Repeated Measurements*

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>df/dt</th>
<th>MS</th>
<th>F</th>
<th>TTP</th>
<th>−dF/dt</th>
<th>MS</th>
<th>F</th>
<th>df/dt</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>7</td>
<td>15.39</td>
<td>6.26</td>
<td>27.51</td>
<td>13.89</td>
<td>1.12</td>
<td>28.01</td>
<td>7.03</td>
<td>3.70</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error(ISO)</td>
<td>16</td>
<td>2.45</td>
<td>1.98</td>
<td>0.04</td>
<td>1.90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within</td>
<td>72</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISO</td>
<td>4</td>
<td>2.85</td>
<td>95.0</td>
<td>2.16</td>
<td>108</td>
<td>0.10</td>
<td>16.7</td>
<td>3.56</td>
<td>119</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISO X K</td>
<td>4</td>
<td>0.67</td>
<td>22.3</td>
<td>0.16</td>
<td>8.00</td>
<td>0.22</td>
<td>36.7</td>
<td>0.91</td>
<td>30.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error(ISO X K)</td>
<td>64</td>
<td>0.03</td>
<td>0.02</td>
<td>0.006</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Data were transformed to natural logarithms to maintain homogeneity of variance. Ten muscles were studied in 4 mM KCl, 8 in 22 mM KCl. Each muscle was exposed to all concentrations of isoproterenol (0, 3, 10, 30, and 100 nM).

† P < 0.06.
Discussion), this in turn, suggested that the mechanism of excitation-contraction coupling might be altered by the elevated K.

Changes in the mechanism of excitation-contraction coupling were studied indirectly by examining the process of mechanical repriming in muscles bathed in both 4 and 22 mM KCl. In muscles bathed in 4 mM KCl, force of contraction accompanying very early extrasystoles was markedly less than the force of contraction for the basal rate of stimulation (0.2 Hz) (Fig. 5). As the interval between the basic drive and the test stimulus was increased, force of contraction increased as well, reaching a maximum with a stimulus interval of approximately 900 msec and then falling slightly over the next 9 seconds. In contrast, for muscles bathed in 22 mM KCl, the earliest extrasystolic contraction was greater than the force of contraction at the basal rate (Fig. 5); force of the extrasystolic contraction increased with increasing stimulus delay, again reaching a maximum with a stimulus interval of approximately 900 msec, but increasing stimulus delay beyond about 1 second caused a rapid decline in force of the accompanying contraction; this decline continued

### Table 3: Electrophysiological Effects of Isoproterenol

<table>
<thead>
<tr>
<th></th>
<th>Isoproterenol concentration (nm)</th>
<th>ANOVA*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td><strong>A [K]_o = 4 mm (n = 4)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RP (mV)</td>
<td>-78</td>
<td>-77</td>
</tr>
<tr>
<td>Overshoot (mV)</td>
<td>24</td>
<td>26</td>
</tr>
<tr>
<td>Plateau amplitude (mV)</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>dV/dtmax (V/sec)</td>
<td>237</td>
<td>247</td>
</tr>
<tr>
<td>APD40 (msec)</td>
<td>192</td>
<td>192</td>
</tr>
<tr>
<td>APD90 (msec)</td>
<td>255</td>
<td>255</td>
</tr>
<tr>
<td><strong>B [K]_o = 22 mm (n = 4)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RP (mV)</td>
<td>-45</td>
<td>-45</td>
</tr>
<tr>
<td>Action potential amplitude (mV)</td>
<td>72</td>
<td>74</td>
</tr>
<tr>
<td>dV/dtmax (V/sec)</td>
<td>6.4</td>
<td>7.4</td>
</tr>
<tr>
<td>APD90 (msec)</td>
<td>211</td>
<td>235</td>
</tr>
</tbody>
</table>

In part B, since only one rising phase of the action potential is seen, action potential amplitude was measured, rather than overshoot or plateau amplitude; APD40 was not measured.

* ANOVA shows the mean square (MS) value for the natural logarithm of the variable analyzed.

† P < 0.05 by ANOVA (4, 12 df).
for delays longer than the basic driving rate. Not shown in this figure is the response of the muscle to the next regular stimulus after the extrasystole. For muscles bathed in 4 mM KCl, the next regular beat was markedly potentiated (postextrasystolic potentiation), and the degree of this potentiation was inversely related to the degree of prematurity of the test stimulus (longer delays evoked less potentiation than shorter delays). Muscles bathed in 22 mM KCl, on the other hand, showed no evidence of postextrasystolic potentiation following any premature stimulus; force of the next regular beat was always equal to that of the beat preceding the extrasystole.

**Discussion**

The most important effects of isoproterenol described in this report concern not its effects on force contraction but its effects on time-to-peak force. These actions can be divided into two groups: (1) in the presence of normal [K]o, isoproterenol has a biphasic effect, increasing time-to-peak force with low concentrations and decreasing TTP as the concentration is increased; (2) in the presence of elevated [K]o, isoproterenol increases time-to-peak force throughout the concentration range tested.

It is generally believed that the positive inotropic actions of isoproterenol result from an increase in Ca influx via the slow inward current, coupled with an increase in the ability of intracellular organelles, notably the sarcoplasmic reticulum, to handle the increased Ca load. Thus, not only is more Ca admitted into the cell during each contraction cycle, but there is a greater amount of Ca in releasable intracellular stores to contribute Ca for contraction (for review, see Scholz, 1980). In the contraction waveform, the effect of isoproterenol to augment Ca metabolism by intracellular organelles is thought to explain both the decrease in the time-to-peak force caused by the drug and an increase in the relative rate of relaxation. In the present study, "relaxing effect" of isoproterenol was seen only in terms of a decrease in time-to-peak force; the relative rate of relaxation was not consistently altered by the drug. This, however, probably is a result of the temperature and stimulation rate used, as relaxing effects of β-adrenergic amines are more pronounced at lower temperatures and faster rate of stimulation (Morad and Rolett, 1972). The increase in time-to-peak force seen in muscles bathed in 4 mM KCl with isoproterenol concentrations near threshold (3 nM) was unexpected. Such a separation between inotropic actions and effects on time-to-peak force might suggest that the two phenomena are not related. It has been shown, for example, that the effect of isoproterenol to enhance the rate of automaticity in canine cardiac Purkinje fibers involves a different receptor interaction than does the effect of isoproterenol to increase the amplitude of the action potential plateau (Grabowski et al., 1978). Alternatively, the increase in time-to-peak force seen with 3 nM isoproterenol might result from an α-adrenergic action of the amine superimposed on its more familiar β-adrenergic action. α-adrenergic agonist actions of isoproterenol have been reported in canine cardiac Purkinje fibers (Rosen et al., 1977), cat nictitating membrane (Trendelenburg, 1974), and mosquito antennae (Nijhout and Martin, 1978). The finding that the α-adrenergic antagonist phentolamine blocks the increase in time-to-peak force, but not the positive inotropic action of isoproterenol, suggests that the increase in time-to-peak force is indeed α-adrenergic action of isoproterenol.

The effect of isoproterenol to increase time-to-peak force in muscles bathed in 22 mM KCl is not so readily explained, since it is not blocked by phentolamine and is also seen when 3-isobutyl-1-methyl-xanthine is used in place of isoproterenol, making it unlikely that this is an α-adrenergic action of isoproterenol. This dramatic increase in time-to-peak force was surprising, as Thyrum (1974) has shown, for guinea pig atrial muscle studied under similar conditions, that isoproterenol causes little or no change in action potential duration or time-to-peak force, and Ingebritsen et al. (1977) reported that 1 μM isoproterenol causes the same changes in force, rates of force development and relaxation, and time-to-peak force in guinea pig ventricular muscle bathed in either 3.6 or 22 mM KCl. However, a later report from the same laboratory (Becker et al., 1977) clearly shows a concentration-dependent increase in time-to-peak force with isoproterenol in guinea pig ventricular muscle bathed in 22 mM KCl. The positive inotropic effect of isoproterenol in muscles bathed in 22 mM KCl is very closely linked to the effect of the drug on time-to-peak force, which in turn is correlated very highly with the
effect of the drug to increase the duration of the slow response action potential (Fig. 4). That is, increasing the duration of depolarization increases the time in which the muscle is able to develop force. However, this is not a normal property of mammalian cardiac muscle. Voltage clamp studies have shown repeatedly that increasing the duration of depolarization may increase force of contraction, but it has no effect on time-to-peak force; the time course of contraction remains unchanged (Beeler and Reuter, 1970; Morad and Goldman, 1972; New and Trautwein, 1972). Thus, the normal relationship between membrane potential and the ability of the muscle to contract seems to be altered in muscles partially depolarized by 22 mM KCl. Since the normal process of excitation-contraction coupling in mammalian myocardium is thought to involve primarily a release of Ca from intracellular stores (Fabiato and Fabiato, 1978), it is tempting to speculate that, for muscles bathed in 22 mM KCl, the coupling mechanism itself is altered, allowing the muscle to utilize Ca from extracellular sources directly for contraction. The reasons for this are of necessity indirect, but rest on three key points:

1. Contraction proceeds for the duration of membrane depolarization, as outlined above. Although the relationship between membrane potential and contraction in mammalian myocardium is a complex one, it is highly unusual to find positive inotropic agents which increase action potential duration (Reiter, 1972), and even more rare to find conditions in which action potential duration is the apparent controlling factor in determining the duration of the contraction. Most interventions which increase action potential duration and time-to-peak force, such as a decrease in the rate of stimulation or a decrease in extracellular Ca, are associated with a fall in force of contraction (see, for example, Allen et al., 1976). Drugs, such as the methylxanthines, which increase force, time-to-peak force, and action potential duration together are generally thought to interfere with sarcoplasmic reticulum function (Reiter, 1972).

2. There is no evidence for postextrasystolic potentiation in muscles bathed in 22 mM KCl. Postextrasystolic potentiation is thought to involve filling of intracellular Ca stores during the extrasystole, providing more Ca for release in the subsequent contraction (Morad and Goldman, 1972).

3. Although the kinetics of mechanical repriming are essentially the same in the two K concentrations (Fig. 4), the absolute magnitude of the force of the premature beat is markedly enhanced in muscles bathed in 22 mM KCl. Mechanical repriming is thought to reflect the time needed for Ca in intracellular sites to circulate from the point of sequestration to a release site, from which it can be released in the next contraction cycle. A critical point in this hypothesis is that the release site is largely, although not necessarily completely, depleted of Ca by the release process, so that early extrasystoles are very weak compared to the preceding contraction. In contrast, when muscles are bathed in 22 mM KCl, early extrasystoles are much stronger than the preceding contraction, and force of contraction declines rapidly with progressively longer delay of the extrasystolic stimulus. The events seen in the early portion of the repriming process strongly suggest either that Ca can somehow circulate within the cell much more rapidly when the cell is bathed in 22 mM KCl or that an additional component is summing with whatever Ca is released from intracellular sites. However, the process may be complicated by the sustained depolarization of the membrane, which might be expected to alter the time course of Ca influx from the cell (Mullins, 1979). If this is the case, the magnitude of the repriming process may simply reflect a time-dependent Ca overload within the sequestering system, an overload which resolves itself during the usual interstimulus interval.

The inotropic pattern of isoproterenol in cat ventricular muscle bathed in 22 mM KCl resembles the effects of isoproterenol and other β-adrenergic amines in frog myocardium bathed in normal [K] (Niedergerke and Page, 1977). Frog myocardium is generally thought to utilize extracellular Ca for contraction (for review, see Morad and Goldman, 1972), again suggesting that extracellular Ca may play an important role in cat ventricular muscle under these conditions.

In any event, although the details are not yet clear, it is evident that the processes underlying the positive inotropic effect of isoproterenol in cat ventricular muscle change when the muscle is bathed in solutions of differing extracellular K. It is unlikely that this shift represents a change from β-adrenergic activation to α-adrenergic activation. The data are consistent with a model of excitation-contraction coupling in which extracellular Ca stores are of primary importance for contraction in muscles bathed in 22 mM KC1 but intracellular Ca stores are of primary importance in muscles bathed in 4 mM KC1; however further studies are required to examine the function of intracellular organelles to bear this out.

Acknowledgments

I thank Lucy Doyle for her excellent technical assistance.

References


Bruckner R, Hackbart I, Meinitz T, Schmelze B, Scholz H (1976) The positive inotropic effect of phenylephrine in the presence of propranolol. Increase in time to peak force and
in relaxation time without increase in cAMP. Naunyn
Schmiedebergs Arch Pharmacol 303: 205-211

Brutsaert DL, DeClerck NM, Goethals MA, Houmansa PR
(1973) Relaxation of cardiac muscle. J Physiol (Lond) 283:
469-486

Cranefield PF (1975) The conduction of the cardiac impulse.
Mount Kisco, New York, Futura Publishing Co.

Fabriato A, Fabriato F (1978) Calcium-induced release of calcium
from the sarcoplasmic reticulum of skinned cells from adult
human, dog, cat, rabbit, rat and frog hearts and from fetal and

Grabowski W, Luttgau H Ch, Schulze JJ (1978) The effects of
isoprenaline and a new β-sympathomimetic amine upon sponta-
aneous activity, diastolic depolarization and plateau height in
cardiac Purkinje fibres. Br J Pharmacol 65: 427-434

Ingebritsen WR Jr, Becker E, Friedman WF, Mayer SE (1977)
Contractile and biochemical responses of cardiac and skeletal
muscle to isoproterenol covalently linked to glass beads. Circ
Res 40: 474-484

Morad M, Goldmann Y (1972) Excitation-contraction coupling
in heart muscle. Membrane control of development of tension.
Prog Biophys Mol Biol 27: 257-313

Morad M, Roletti EL (1973) Relaxing effects of catecholamines
on mammalian heart. J Physiol (Lond) 224: 537-558

Mullins W (1979) The generation of electric currents in cardiac
fibers by Na/Ca exchange. Am J Physiol 236: C103-C110

Myers JL (1979) Fundamentals of Experimental Design, ed. 3,
Boston, Allyn and Bacon

New W, Trautwein W (1972) The ionic nature of slow inward
current and its relation to contraction. Pflugers Arch 334:
24-38

Niedergerke R, Page S (1977) Analysis of catecholamine effects
in single atrial trabeculae of the frog heart. Proc R Soc (Lond)
[Biol] 197: 333-362

Nijhout HF, Martin SK (1978) Alpha-adrenergic activity of
isoproterenol in mosquito antennae. Experientia 34: 768-778

adrenergic amines on electrophysiological properties and au-
tomacity of neonatal and adult canine Purkinje fibers. Circ
Res 40: 290-400

Scholz H (1980) Effects of beta- and alpha-adrenoceptor activa-
tors and adrenergic transmitter releasing agents on the me-
chanical activity of the heart. In Handbook of Experimental
Pharmacology, edited by L Szekeres 54/1: Adrenergic activa-
tors and inhibitors, Part I. New York, Springer, pp 651-733

Thyrum PT (1974) Inotropic stimuli and systolic transmembrane
calcium flow in depolarized guinea-pig atria. J Pharmacol Exp
Ther 188: 166-179

Trendelenburg U (1974) An analysis of the alpha-and beta-
effects of isoprenaline on the isolated nictitating membrane
Naunyn Schmiedebergs Arch Pharmacol 285: 375-393

Watanabe AM, Bech HR Jr (1974) Cyclic adenosine mono-
phosphate modulation of slow calcium influx channels in
guinea pig hearts. Circ Res 35: 316-324

Wiggins JR (1980a) Inotropic actions of isoproterenol in cat
ventricular muscle: Effects of extracellular potassium (abstr).
Physiologist 23: 56

Wiggins JR (1980b) Inotropic actions of isoproterenol in cat
ventricular muscle: Effects of pretreatment with insecticide
(abstr). Pharmacologist 22: 286
Inotropic actions of isoproterenol in cat ventricular muscle. Effects of extracellular potassium.
J R Wiggins

doi: 10.1161/01.RES.49.3.718

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1981 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/49/3/718