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Actions of Adenosine and Isoproterenol on Isolated Mammalian Ventricular Myocytes

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SUMMARY. We investigated the effects of adenosine and isoproterenol on enzymatically dispersed ventricular myocytes from bovine and guinea pig hearts. Intracellular stimulation of relaxed myocytes with regular striation patterns and normal resting potential resulted in action potentials with full plateaus accompanied by contractions. Adenosine in concentrations up to 0.2 mM had no significant effect on any of the action potential parameters or on the basal contractility. In contrast, in the same cells, adenosine effectively antagonized the stimulatory effect of isoproterenol. Isoproterenol (1–10 nM) prolonged the action potentials by 34–41%, displaced the plateau to more positive potentials, and caused a 3-fold increase in the extent of myocyte sarcomere shortening. In the presence of adenosine (5–50 μM), isoproterenol increased the action potential duration by only 8–9%, the shift of the plateau was nearly abolished, and the increase in the extent of myocyte sarcomere shortening was less than 10%. In some of the myocytes, isoproterenol (1–10 nM) induced depolarizing afterpotentials accompanied by aftercontractions. The afterdepolarizations occasionally reached threshold resulting in triggered sustained rhythmic activity. Adenosine (20–50 μM) not only reduced the amplitude of the afterdepolarizations and aftercontractions, but also abolished the sustained rhythmic activity. We conclude, first, that isolated ventricular myocytes respond to isoproterenol and adenosine; second, that adenosine has no direct effect, but effectively antagonizes the stimulatory actions of isoproterenol; third, that findings are consistent with the ones reported for multicellular ventricular preparations; fourth, that adenosine concentrations required to attenuate the actions of isoproterenol are in the range of adenosine concentrations released by cardiac cells when oxygen availability is limited and/or demand is increased; and fifth, that endogenously released adenosine may modulate the electrophysiological and contractile effects of catecholamines. (*Circ Res 53: 287–297, 1983*)

RECENTLY, adenosine has been recognized to antagonize specifically the inotropic, and some of the electrophysiological and metabolic, effects of catecholamines in ventricular myocardium (Schrader et al., 1977; Dobson, 1978; Baumann et al., 1981, Belardinelli et al., 1982).

The observation that adenosine antagonizes the inotropic effect of tyramine, but not the tyramine-induced release of norepinephrine, has been assumed to indicate that adenosine acts postsynaptically (Schrader et al., 1979). However, adenosine has also been shown to inhibit the stimulation-induced release of norepinephrine from sympathetic nerve terminals in the heart (Hedquist and Fredholm, 1976; Wakade and Wakade, 1978). Consistent with this latter finding, in the dog, adenosine has been shown to reduce the increase in heart rate induced by electric stimulation of the right stellate ganglion (Hom and Lokhandwala, 1981). These observations suggest that the antagonism between adenosine and sympathetic nerve stimulation in the heart is due, at least in part, to the inhibitory effect of adenosine on the release of norepinephrine from adrenergic nerve endings. Thus, in the intact heart, it seems that adenosine can act both prejunctionally, at the level of adrenergic nerve terminals, and postjunctionally, at the level of the cardiac cells. The postjunctional effects of adenosine can be better investigated in the isolated cells since they are free of hormonal influences and devoid of nerve terminals (Fry et al., 1979; Dow et al., 1981).

The purpose of the present study was to characterize the postjunctional antagonistic action of adenosine on the electrophysiological and contractile effects of isoproterenol in isolated mammalian ventricular myocytes.

Methods

Cell Preparation

Single isolated myocytes were prepared from the ventricles of adult guinea pigs and bovine hearts by enzymatic

dissociation (Glick et al., 1974; Dow et al., 1981). The ventricular myocytes of the guinea pig hearts were enzymatically isolated by retrograde perfusion of the aorta ("perfusion method"), whereas, the myocytes of bovine hearts were isolated by incubating ventricular tissue chunks in enzyme medium ("chunk method") (Isenberg and Klockner, 1982a).

Perfusion Method

The guinea pig hearts were perfused for 5 mintues with an oxygenated (100% O_2), prewarmed (35°C) "low Ca^{++} medium" (pCa = 5.5) containing in mM: 120 NaCl, 10.8 KCl, 5 $MgSO_4$, 5 Na-pyruvate, 20 glucose, 20 taurine, adjusted with 5 mM Aces/NaOH to pH 6.9. Thereafter, the perfusate was switched to the "enzyme medium" that was the same low Ca^{++} medium plus 40 μM Ca^{++} to activate the collagenase (1 mg/ml, Sigma type I). After perfusion with the enzyme medium for 10–15 minutes, the hearts were perfused with 50 ml of a medium containing in mM: 85 KCl, 30 K_2HPO_4, 5 $MgSO_4$, 5 Na_2ATP, 5 pyruvic acid, 5 β-hydroxy butyric acid, 20 taurine, 20 glucose, and 50 g/liter polivinyl pyrollidone (PVP 40), pH 7.2, and pCa 7.6, i.e., the "KB" medium (Isenberg and Klockner, 1980, 1982a). The ventricles were cut out and chopped with scissors. The cells were released from the chunks by mechanical agitation and stored in KB medium for at least 1 hour before exposure to calcium-containing solutions. This 1 hour preincubation of the myocytes in KB medium results in a greater yield of Ca-tolerant cells (Isenberg and Klockner, 1982a). The guinea pig ventricular myocytes measured 98 ± 11 μm in length and 22 ± 6 μm in diameter (n = 200) (Isenberg and Klockner, 1982a).

Chunk Method

Left ventricular tissue chunks (2 mm thick) from bovine hearts were stirred (with O_2 bubbles) in low Ca^{++} medium at room temperature for 30 minutes. Afterward the chunks were incubated in the enzyme medium at 35°C (same medium as above but complemented with 1 mg/ml hyaluronidase, Sigma). After 20 minutes, the supernatant containing the dissociated cells was separated from the chunks by passing it through a 200-μm mesh net into the KB medium. The chunks were reexposed to fresh enzyme medium for three additional 20-minute periods. The cells were stored in the KB medium for at least 1 hour prior to the experiments. The bovine ventricular myocytes measured 136 ± 21 μm in length and 35 ± 10 μm in diameter (n = 200) (Isenberg and Klockner, 1982a).

Chamber and Solutions

KB medium containing the isolated cells was pipetted into the experimental chamber. The cells were continuously superfused (2–3 ml/min) with Tyrode's solution (pH 7.4, 35 ± 1°C) with the following composition in mM: NaCl 150, KCl 5.4, $MgCl_2$ 1.2, $CaCl_2$ 3.6, Hepes 5, glucose 10. The time course for changing solutions (e.g., from normal K^+ to high K^+) is illustrated in Figure 1. Changes in $[K^+]_o$ were frequently made to ascertain the adequacy of the superfusion of the cell under investigation.

The prewarmed solutions were not gassed to avoid formation of gas bubbles; gas bubbles can push the cells against the microelectrode tip, resulting in mechanical damage of the cells. Comparative experiments utilizing gassed and ungassed solutions revealed no differences in Ca tolerance, electrophysiological parameters, or ability of the myocytes to contract (Isenberg and Klockner, 1982a).

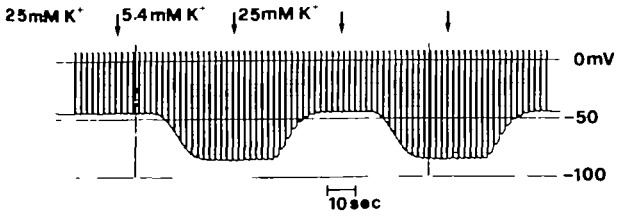

FIGURE 1. *Effect of changes in extracellular potassium $[K^+]_o$ on the membrane resting potential of an isolated guinea pig ventricular myocyte. The arrows indicate the point at which the change in solution was made. The time delay in response to switching from 25 mM $[K^+]_o$ to 5.4 mM $[K^+]_o$ (first arrow from the left) was due to the dead space created by the tubing connections (e.g., it took 12–13 sec before the cell started to hyperpolarize). The time course in response to increasing $[K^+]_o$ from 5.4 to 2.5 mM was similar (second arrow from the left)*

Drugs

Isoproterenol-HCl (Sigma) and adenosine (Sigma) were dissolved in Tyrode's solution of the same composition as the one used to superfuse the cells. To prevent the oxidation of isoproterenol, all the solutions contained 30 μM Na_2EDTA.

Electrophysiological Techniques

The membrane resting and action potentials were recorded with conventional microelectrodes as described by Isenberg and Klockner (1982a). The cells were stimulated at a rate of 0.5 Hz with current pulses 1 msec in duration and an amplitude of less than 10 nA.

Contractility

In response to electrical stimulation, the single isolated myocytes shorten and relengthen. This type of unloaded contraction was studied with a TV camera-tape system adapted to an inverted microscope, as previously described (Isenberg, 1982). In brief, the cell image on the monitor was magnified 2,600 times, using a Zeiss LD 40 objective. The movement of the cell edge on the TV monitor was converted to an analog signal by means of an array of 50 photodiodes. Off line, the signal was calibrated in terms of the average sarcomere length that was evaluated from single images on the TV tape. From the recorded signals, the extent and time course of the unloaded shortening of the myocytes were estimated. Upon electrical stimulation, the myocytes shorten from their maximum diastolic length (SL^{diast}) to a minimum systolic length (SL^{syst}). The extent of shortening was calculated according to $(SL^{diast} - SL^{syst})/SL^{diast}$. For example, for a SL^{diast} of 1.85 μm, and SL^{syst} of 1.65 μm, the calculated extent of shortening is 0.12. To characterize the time course of the myocyte shortening, the time to peak of shortening (TTP) was defined as the period between stimulus and SL^{syst}. In addition, the rate of sarcomere shortening and relengthening was calculated from the $\Delta SL/20$ msec (the TV system employed has a 50 Hz image frequency which allows one sampling point every 20 msec); the normalized rate of shortening was defined as rate of SL shortening divided by $SL^{diast} - SL^{syst}$.

Limitations of the Method Employed for Assessment of Contractility

The TV system used has a 50 Hz image frequency, and, thus, the maximal number of data points that can be

obtained per second for measurement of SL is 50, i.e., one every 20 msec. For instance, the time course of shortening in myocytes with a time to peak (TTP) of 120 msec (e.g., guinea pig) is described by 6 data points whereas by 12 data points in myocytes with a TTP of 240 msec (e.g., bovine). Therefore, the SL^{syst} and the extent of shortening are underestimated (maximum estimated error of 4%). The rate of shortening and relengthening are also underestimated (maximum 10%).

The continuous pen record depicted in Figure 5 shows some degree of drift on the baseline which can result from shadows in the TV monitor due to changes in the meniscus surrounding the microelectrode, air bubbles, or debris floating in the medium. To minimize the drift problems, frequent off-line calibrations were made from single images of the TV tape.

Contractile force was not measured, and, hence, a direct comparison of the present results with the contractility data of the literature is not possible. However, a recent study in single cardiac cells has shown that changes in mechanical parameters reflect changes in sarcomere length (De Clerck et al., 1981). Thus, we feel that the unloaded shortening of the myocytes here studied give useful information for at least qualitative comparisons.

Data Analysis and Statistics

The action potentials (APs) and contractions were displayed on a storage oscilloscope (Tektronix 5103N) and on a two-channel strip-chart recorder (Gould Brush model 220). The data were digitized (10 bit resolution) and stored on a digital tape recorder (Macrodyn, ERDAC 4500). Off line, the stored data were analyzed with a Hewlett-Packard 85 microcomputer. The computer was programmed to evaluate the resting potential (RP), amplitude (Ampl), overshoot (OV), duration as measured at 90% (APD_{90}) and at 50% (APD_{50}) of the peak action potential amplitude, and maximal upstroke velocity ($+\dot{V}_{max}$). The APs and contractions were plotted using a Hewlett-Packard plotter, model 7225 A, for illustration purposes.

All data are expressed as mean ± SEM. The mean values reported are derived from the numer of cells (n) studied to which any particular animal has contributed with a maximum of two cells. Statistical analysis was based upon paired "t" distribution for comparisons between control data and all interventions. Comparisons were also made between isoproterenol alone, as well as isoproterenol plus adenosine. The differences were considered to be significant when P was less than 0.05.

Results

The data reported here were obtained from rod-shaped myocytes with regular striation pattern and normal resting potential that—upon intracellular stimulation—gave rise to action potentials with full plateaus. In normal Tyrode's solution (5.4 mM K^+ and 3.6 mM Ca^{++}), the resting potential was −76.5 ± 1.5 mV for the bovine cells (n = 10) and −87.2 ± 2.0 mV for the guinea pig ventricular myocytes (n = 12). The action potential duration (APD_{90}) was longer in bovine cells than in guinea pig, i.e., 251 ± 2 msec (n = 9) for the bovine and 158 ± 12 ms (n = 10) for the guinea pig cells. The maximal upstroke velocity ($+\dot{V}_{max}$) was 210 ± 31 V/sec (n = 9) in guinea pig cells and 254 ± 27 V/sec (n = 10) in the bovine myocytes (for comparison, see Isenberg and Klockner, 1982a). Some of the myocytes responded with depolarizing afterpotentials and aftercontractions. These myocytes were studied as a separate group (see below).

Lack of Direct Effect of Adenosine

Exposure of the myocytes to adenosine in concentrations up to 0.2 mM resulted in no significant change in resting potential, action potential configuration or contractility (Fig. 2). Similar results were observed in four bovine and three guinea pig cells. For example, in bovine myocytes, the control ADP_{90} was 242 ± 20 msec, and after 0.2 mM adenosine, was 236 ± 17 msec. Adenosine caused no change in resting potential of either species; neither did it modify the maximal upstroke velocity.

Effects of Isoproterenol

As illustrated in Figure 3, isoproterenol caused significant changes in the action potential configuration, both in bovine and guinea pig ventricular myocytes. Isoproterenol increased the action potential duration (APD_{50} and APD_{90}) and displaced the overshoot and the plateau to more positive potentials. In bovine cells, a small hyperpolarization (up to 4 mV) was observed. The effects of isoproterenol are summarized in Tables 1 and 2. Comparing the two species, the response of the bovine ventricular myocytes to isoproterenol was greater than the myocytes from guinea pigs. In bovine ventricular myocytes, 1 nM isoproterenol prolonged the APD_{50} and APD_{90} by 41 and 39%, whereas, in guinea pig myocytes, 1 nM of isoproterenol were required to prolong the APD_{50} and APD_{90} by 37 and 34%, respectively. Thus, in order to obtain clear and reproducible effects, isoproterenol was applied at 1nM to bovine and at 10 nM to guinea pig ventricular myocytes.

Antagonism by Adenosine of the Effects of Isoproterenol

Adenosine in concentrations ranging from 5 to 0.2 mM significantly antagonized the effects of isoproterenol on the action potentials in bovine (Fig. 3, panel A) and guinea pig ventricular myocytes (Fig. 3, panel B). When the action potential in bovine cells was enhanced by 0.1 nM isoproterenol, concentrations of adenosine as low as 5 μM were sufficient to antagonize the effects of isoproterenol completely. In the presence of 1 nM isoproterenol, the antagonism required higher concentrations of adenosine, and sometimes, it remained incomplete. As summarized in Table 1 (bovine), in the presence of 50 μM adenosine, 1 nM isoproterenol increased the APD_{50} and APD_{90} by only 8.5% and 11.4%, respectively. Thus, the effect of isoproterenol was largely, but not completely, inhibited. In the guinea pig cells (Table 2) the prolongation of the action potential caused by 10 nM isoproterenol was also markedly, but not completely, attenuated by 0.2 mM adenosine;

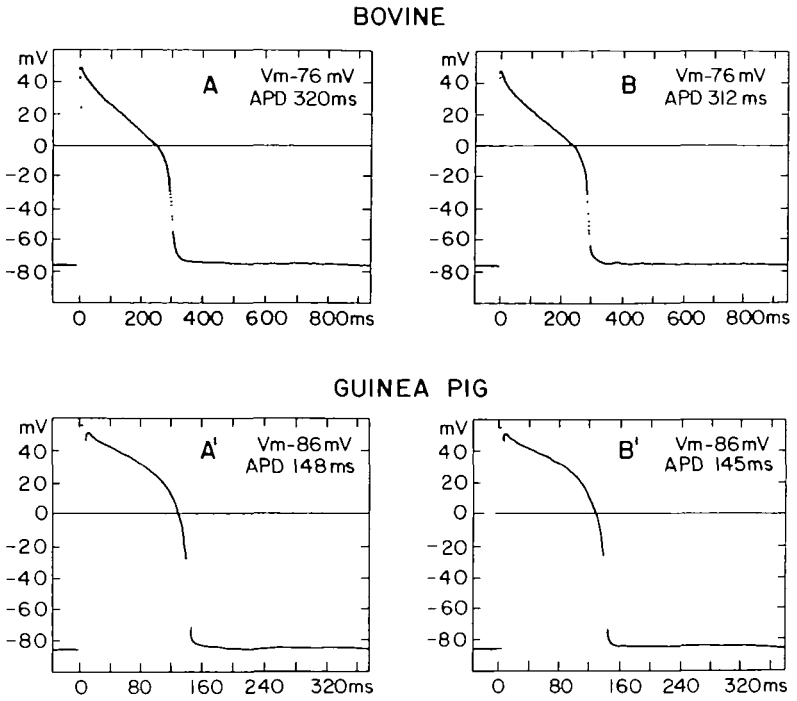

FIGURE 2. *Lack of effect of adenosine (ADO) on the action potentials (APs) of isolated ventricular myocytes from bovine and guinea pig hearts. Panels A and A' illustrate the control APs Panels B and B' were obtained 2 minutes after exposure of the myocytes to 0.2 mM ADO The slight shortening of the APs (8 msec in panel B and 3 msec in panel B') is not significant.*

that is, the increase in APD_{50} and APD_{90} was only 9.3 and 8.6%, respectively. In addition, adenosine also antagonized or attenuated the effects of isoproterenol on the overshoot and resting potential (Tables 1 and 2). After the washout of isoproterenol and adenosine, all the action potential parameters returned to values no different from control (Tables 1 and 2).

Contractility

The actions of adenosine on the contractile effects of isoproterenol and elevated $[Ca^{++}]_o$ were studied in bovine and guinea pig ventricular myocytes. Contractility in the unloaded isolated myocytes was evaluated by estimating the extent and rate of shortening of the myocytes. The results obtained from bovine ventricular myocytes are summarized in Table 3. Exposure of the myocytes to 1 nM isoproterenol caused a significant increase in the extent of the myocytes shortening (i.e., from 0.12 ± 0.03 to 0.24 ± 0.025, Table 3). Addition of 50 μM adenosine to the isoproterenol containing medium resulted in a significant reduction in the extent of shortening (Table 3). That is, adenosine antagonized the isoproterenol-induced increase in the extent of shortening. Isoproterenol also increased the rate of sarcomere

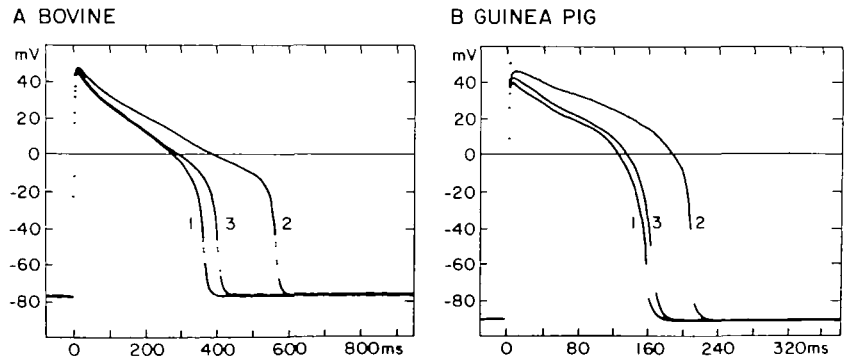

FIGURE 3. *Effect of adenosine (ADO) on the isoproterenol (ISO)-enhanced action potentials (APs) of isolated ventricular myocytes from bovine (panel A) and guinea pig (panel B) hearts. In both panels, action potential 1 (AP-1) is the control. AP-2. addition of ISO (1 nM, bovine and 10 nM, guinea pig) prolonged the APs, caused an increase in the overshoot, and displaced the plateau to more positive potentials. AP-3· in the continued presence of ISO, ADO (50 μM, bovine and 0.2 mM, guinea pig) inhibited the ISO effects. All records in this and subsequent figures were obtained at steady state effects of the interventions (i.e., 2–3 minutes after addition of ISO and at 2 minutes after ADO).*

TABLE 1
Summary of Data Demonstrating Attenuation by ADO of ISO-Induced Prolongation of the Action Potential in Isolated Bovine Ventricular Myocytes

	Control	ISO (1 nM)	ISO (1 nM) + ADO (50 μM)	Washout
RP	−77.9 ± 1.7	−80.3 ± 0.8	−79 3 ± 1.2	−76.8 ± 1.6
OV	+39.3 ± 2.1	+40.4 ± 2.0	+37.5 ± 2.3	+35.5 ± 2.4
AMPL	117.2 ± 2.9	120.7 ± 2.7	116.8 ± 3 5	112.3 ± 3 1
APD_{50}	203.9 ± 26.9	287.3 ± 25.9*	221.4 ± 31.3†	201.6 ± 42 6
APD_{99}	240 6 ± 25.1	334.6 ± 26.6*	268.1 ± 30 0†	244.3 ± 46 5
n =	6	6	6	6

RP = resting potential; OV = overshoot; AMPL = amplitude in mV; APD_{50} and APD_{90} = action potential duration in milliseconds measured at 50% and 90% of the peak action potential amplitude, respectively. *n* = number of cells. All values are mean ± SEM

* Values significantly different ($P < 0.05$) from control and washout.

† Values significantly different from ISO but not different from control and washout. All the other values were not different from each other.

shortening, and—as in the case of the extent of shortening—adenosine significantly attenuated it (Table 3). On the other hand, the time to peak of shortening (not shown) and the normalized rate of shortening (Table 3) were not significantly altered by isoproterenol, either alone, or in the presence of adenosine.

The antagonism by adenosine on the contractility enhanced by 10 nM isoproterenol in a guinea pig ventricular myocyte is illustrated in Figure 4. When stimulated at low frequencies (0.5 Hz) the guinea pig ventricular myocytes shortened with two distinct components, an early and a late component. As shown in Figure 4, during the first component of contraction, the sarcomere shortened within 120 msec from 1.90 μm (diastole) to 1.80 μm (systole), resulting in a calculated extent of shortening of 0.05. The subsequent relaxation remained incomplete and the second component of contaction shortened the sarcomere to a minimum length of 1.75 μm (extent of shortening of 0.08). Exposure to isoproterenol (e.g., 10 nM) increased the extent of shortening to 0.27, i.e., the minimum systolic length was 1.38 μm. As illustrated in Figure 4, the enhancement of the first component of contraction was greater than the second component (for comparison, see Seibel et al., 1978). When in the continuous presence of 10 nM isoproterenol, 50 μM adenosine was added, the positive inotropic effect of isoproterenol was markedly inhibited. Interestingly, the attenuation of the first component of contraction was greater than the second one. Thus, the contractions measured in the presence of isoproterenol plus adenosine greatly resemble those obtained during control conditions. Similar results were observed in three additional guinea pig ventricular myocytes.

As expected, elevation of extracellular calcium resulted in an increase in the extent by which the myocytes shorten. Figure 5 illustrates the lack of antagonism by adenosine of the positive inotropic effect of calcium. As can be seen, the elevation of $[Ca^{++}]_o$ from 3.6 to 5.4 mM caused a 50% increase in the extent of shortening. Adenosine (0.2 mM) did not attenuate the calcium induced inotropic effect (Fig. 5, row C). In none of the ventricular myocytes studied (four guinea pigs and five bovines) did aden-

TABLE 2
Summary of Data Demonstrating Inhibition by ADO of ISO-Induced Enhancement of the Action Potential in Isolated Guinea Pig Ventricular Myocytes

	Control	ISO (10 nM)	ISO (10 nM) + ADO (0.2 mM)	Washout
RP	−89.8 ± 1.0	−90.2 ± 1.0	−89.7 ± 1.0	−88.7 ± 1.5
OV	+43.8 ± 2.4	+47.5 ± 1 5*	+44.7 ± 1.5†	+45.7 ± 2.8
AMPL	133 6 ± 2.2	137.7 ± 1.0	134.4 ± 1.5	134.4 ± 1.5
APD_{50}	127.7 ± 7.7	174.9 ± 8 0*	139.7 ± 6 2†	137.7 ± 9.7
APD_{90}	138.0 ± 7 0	185.0 ± 8.0*	150.0 ± 7 5†	144.3 ± 8.3
n =	6	6	6	6

Abbreviations: see footnote, Table 1 *n* = number of cells. All values are mean ± SEM.

* Values significantly different ($P < 0.05$) from control and washout.

† Values significantly different from ISO but not different from control and washout All the other values were not different from each other.

TABLE 3
Sarcomere Parameters of Isolated Bovine Ventricular Myocytes Exposed to ISO and ADO

	Control	ISO (1 nM)	ISO (1 nM) + ADO (50 μM)
$SL^{diast}_{(\mu m)}$	1.88 ± 0.03	1.86 ± 0.04	1.86 ± 0.03
$SL^{syst}_{(\mu m)}$	1.65 ± 0.04	1 42 ± 0.05*	1.61 ± 0.04†
Extent of shortening	0.12 ± 0.03	0.23 ± 0.02*	0.13 ± 0.03†
Rate of SL shortening (μm/sec)	−2.3 ± 0.3	−4.5 ± 0.4*	−2.5 ± 0.3†
Normalized rate of shortening (sec^{-1})	−10 ± 0.3	−9.8 ± 0.2	−9.6 ± 0.3
n	7	7	7

Values are mean ± SEM, *n* = nmber of cells.
* Values significantly different ($P < 0.05$) from control.
† Values significantly different from ISO (1 μM), but not from control.

osine depress either the basal contractility or the positive inotropic effect produced by elevation of extracellular calcium.

Delayed Afterdepolarizations and Aftercontractions

In some of the myocytes, depolarizing afterpotentials occurred after the cell had repolarized to the resting potential. Isoproterenol enhanced or even induced such delayed afterdepolarizations in a dose-dependent manner (Figs. 6 and 7; Table 4). The delayed afterdepolarizations were accompanied by aftercontractions (Fig. 8). Amplitude of afterdepolarizations was augmented by increasing the duration of the drive, rate of stimulation, and after a premature stimulus (not illustrated). In the presence

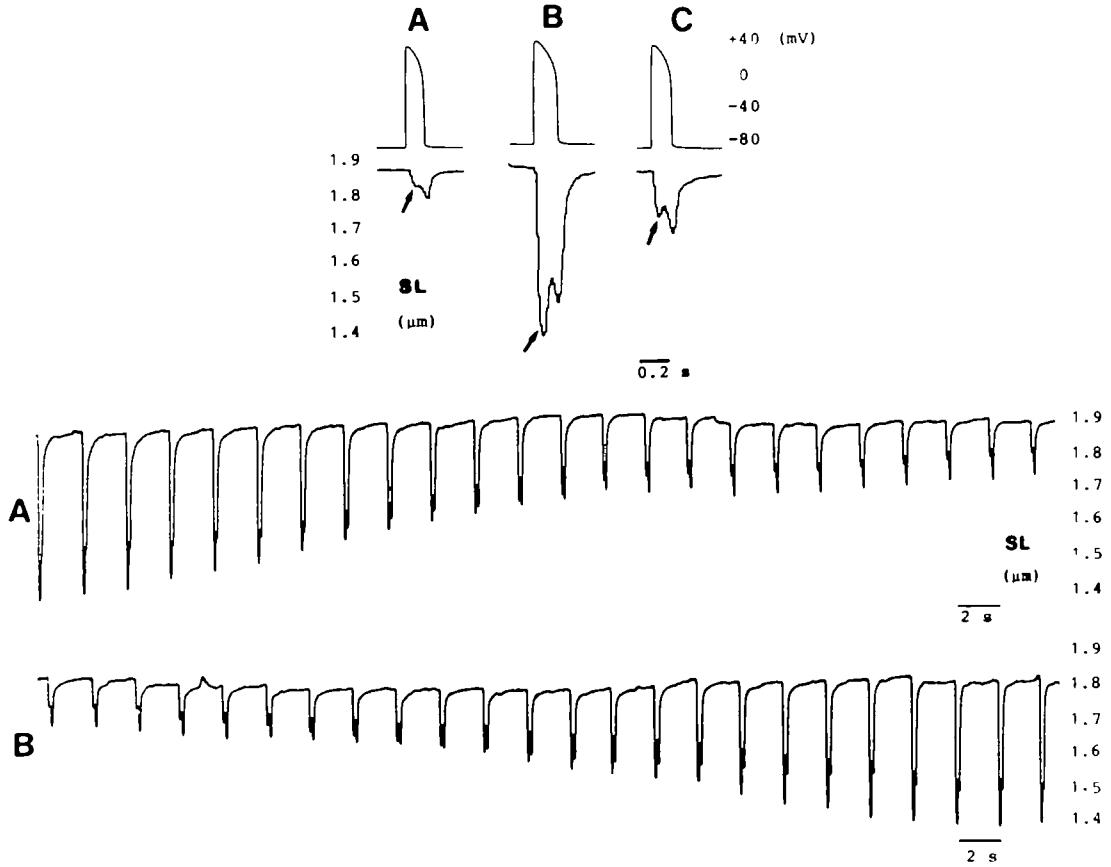

FIGURE 4. *Antagonism by adenosine (ADO) of the isoproterenol (ISO)-induced positive inotropic effect in an isolated guinea pig ventricular myocyte. Contractions measured as shortening of the sarcomere length (SL) are indicated by downward deflections. Upper part depicts three single records of the action potentials and accompanied contractions The arrows point to the "first" component of the sarcomere shortening. Panel A: control; panel B· ISO (10 nM) increased the AP in regard to duration and plateau, and caused a 5-fold increase in the extent of shortening. Also shown is the clear separation of the two components of the contraction. Panel C: addition of 50 μM ADO (in the presence of ISO) markedly attenuated the positive inotropic effect of ISO and shortened the AP to control. Lower part illustrates the time course of the effects of ADO and ISO on the extent of the myocyte shortening. Strip A is the attenuation of the inotropic effect of ISO by 50 μM ADO, and strip B is the washout ADO in the presence of ISO. The illustrated records are from a Brush pen recorder SL is the average sarcomere length.*

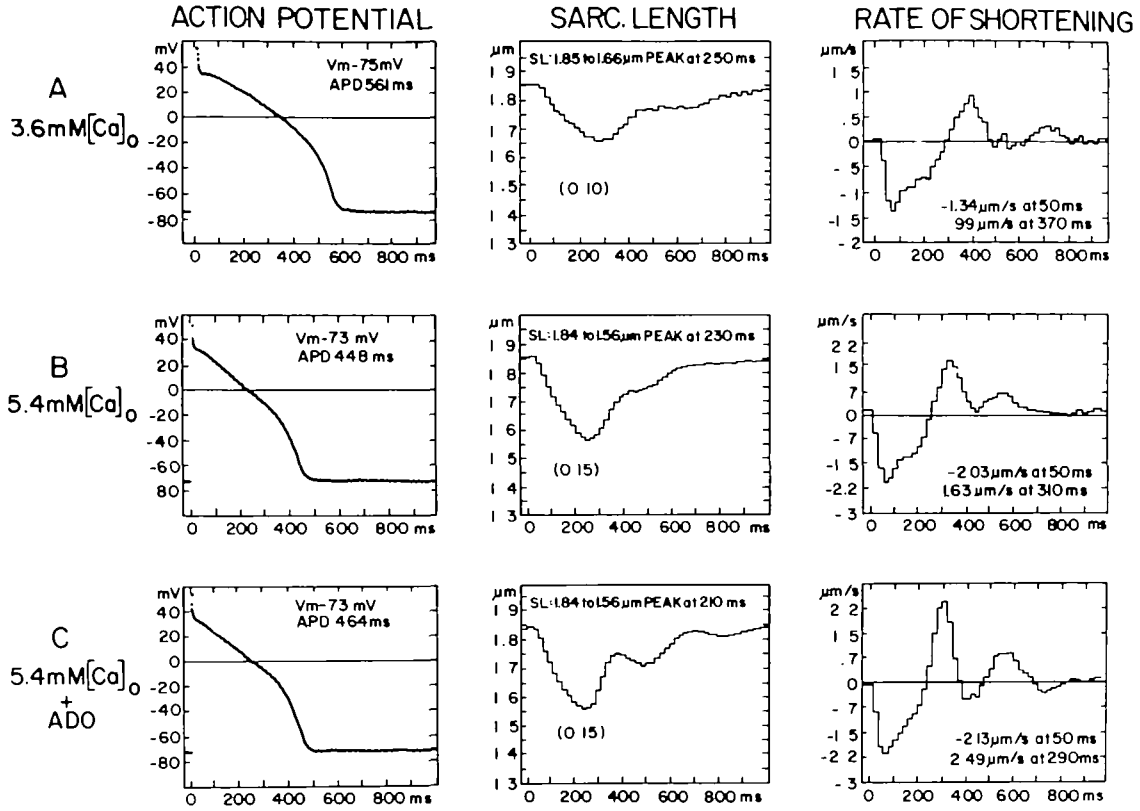

FIGURE 5. *Lack of antagonism by adenosine (ADO) of the calcium-induced shortening of the action potential (AP) and positive inotropic effect in an isolated bovine ventricular myocyte. The myocyte was stimulated at 0.25 Hz. Row A illustrates the control AP, sarcomere length, and rate of shortening obtained in Tyrode's solution containing 3.6 mM $[Ca^{++}]_o$. Row B: 3 minutes after raising $[Ca^{++}]_o$ to 5.4 mM. Row C: 3 minutes after addition of 0.2 mM ADO to the Tyrode's solution containing 5.4 mM $[Ca^{++}]_o$. The resting membrane potential (Vm) and AP duration (APD) measured at 90% of the peak amplitude are shown in the upper righthand corner of each "AP" panel. The sarcomere length (SL) is depicted in the middle column; the diastolic and systolic SL values, as well as time to peak of shortening, are indicated in the upper part of each "SARC Length" panel. The number in parenthesis denotes the calculated extent of shortening (see Methods). The rate of sarcomere shortening and relengthening is depicted in the right column. In each "Rate of Shortening" panel, the maximal rate of shortening is indicated by the negative values, whereas the maximal rate of relengthening is indicated by positive values. As can be seen, elevation of $[Ca^{++}]_o$ increased the extent of shortening from 0.10 (row A) to 0.15 (row B). Addition of 0.2 mM ADO (row C) did not reduce the increase in extent of shortening (0 15) caused by 5 4 mM $[Ca^{++}]_o$. Note that a second component of contraction appeared in 5 4 mM $[Ca^{++}]_o$ (row B), which increased with time and was not reduced by ADO (row C)*

of 1 to 10 nM isoproterenol, nondriven action potentials could arise from the peak of the afterdepolarizations (Fig. 7) and sustained rhythmic activity ensued (Fig. 7), i.e., triggered activity. This sustained rhythmic activity could persist for the entire period of observation (e.g., up to 1 hour) or could be terminated by an extra stimulus. It occasionally ceased spontaneously; however, an extra stimulus or short periods of fast drive could easily reinitiate the sustained rhythmic activity.

TABLE 4
Inhibition by ADO of the ISO-Induced Enhancement of Delayed Afterdepolarizations in Isolated Bovine Ventricular Myocytes

	Amplitude (mV)
Control	1.6 ± 0.5
ISO (1 nM)	4.8 ± 0.2*
ISO (10 nM)	8 6 ± 1.0*
ISO (1 nM) + ADO (20 µM)	2.2 ± 0.6†

Mean ± SEM of the afterdepolarization amplitude from 10 cells.
* Values significantly different ($P < 0.05$) from control
† Values significantly different from ISO (1–10 nM), but not significantly different from control.

In the continued presence of isoproterenol (1–10 nM), adenosine (20–50 µM) markedly reduced the amplitude of the afterdepolarizations (Figs. 6 and 7; Table 4) and terminated the sustained rhythmic activity (Fig. 7). Note in Figure 7 that adenosine in addition to terminating the sustained rhythmic activity also abolished the delay afterdepolarization and shortened the action potential by 90 msec (compare panels A and D). In addition, in the presence of adenosine, either extra stimuli or short periods of fast drive could no longer initiate the sustained rhythmic activity. However, after washout of adenosine, but still in the presence of isoproterenol, the triggered automaticity could be reinitiated by an extra stimulus or by short periods of fast drive.

In five additional myocytes, raising extracellular calcium from 1.8 to 5.4 mM increased the amplitude

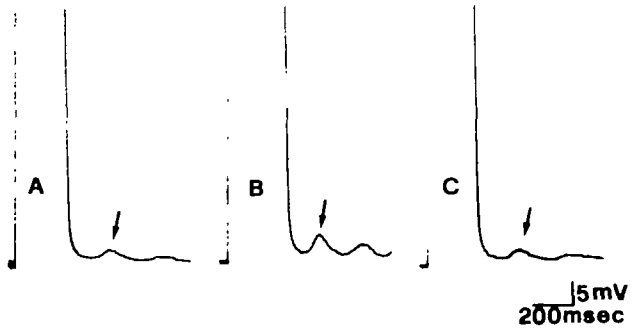

FIGURE 6. *Inhibition by adenosine (ADO) of the isoproterenol (ISO)-induced augmentation of delayed afterdepolarization in an isolated bovine ventricular myocyte Only part of he action potentials are shown. The arrows indicate the delayed afterdepolarization. Panel A: control, panel B. 2 minutes after addition of 1 nM ISO resulted in an increase in the amplitude of the afterdepolarization. Note that a second afterdepolarization is evidenced. Panel C: in the continued presence of ISO, addition of 20 μM ADO antagonized the ISO-induced enhancement of the afterdepolarization.*

of the afterdepolarization from 2–3 mV to 8–10 mV. Addition of adenosine (up to 0.2 mM) did not attenuate the calcium-induced increase in the afterdepolarization.

Discussion

Adenosine has been reported to antagonize the positive inotropic and metabolic effects of catecholamines in ventricular myocardium (Schrader et al., 1977; Dobson, 1978; Baumann et al., 1981). However, in the same tissue (i.e., ventricular myocardium), neither the basal contractile force nor the increase in contractility caused by elevation of extracellular calcium or stimulation of α-adrenoreceptors is depressed by adenosine (Baumann et al., 1981; Endoh and Yamashita, 1980). Such observations have led to the conclusion that the inhibitory effects of adenosine in ventricular myocardium can be demonstrated only under conditions in which the adenylate cyclase-cAMP system of the heart has been previously stimulated (Schrader et al., 1977; Baumann et al., 1981). The present results confirm the findings that adenosine antagonizes the inotropic effect of isoproterenol, but additionally, shows that this antagonism also applies to the electrophysiological effects of isoproterenol. Furthermore, the findings that adenosine in concentrations up to 0.2 mM had no significant effect by itself on the membrane resting and action potential, on basal contractility, as well as on the contractile and electrophysiological effects of elevated $[Ca]_o$, are similar to previous reports in multicellular preparations (Johnson and McKinnon, 1956; De Gubareff and Sleator, 1965). This lack of direct effect of adenosine in ventricular myocardium is in sharp contrast to its direct effect in atrial cells (Johnson and McKinnon, 1956; Belardinelli and Isenberg, 1983), and, as shown in the

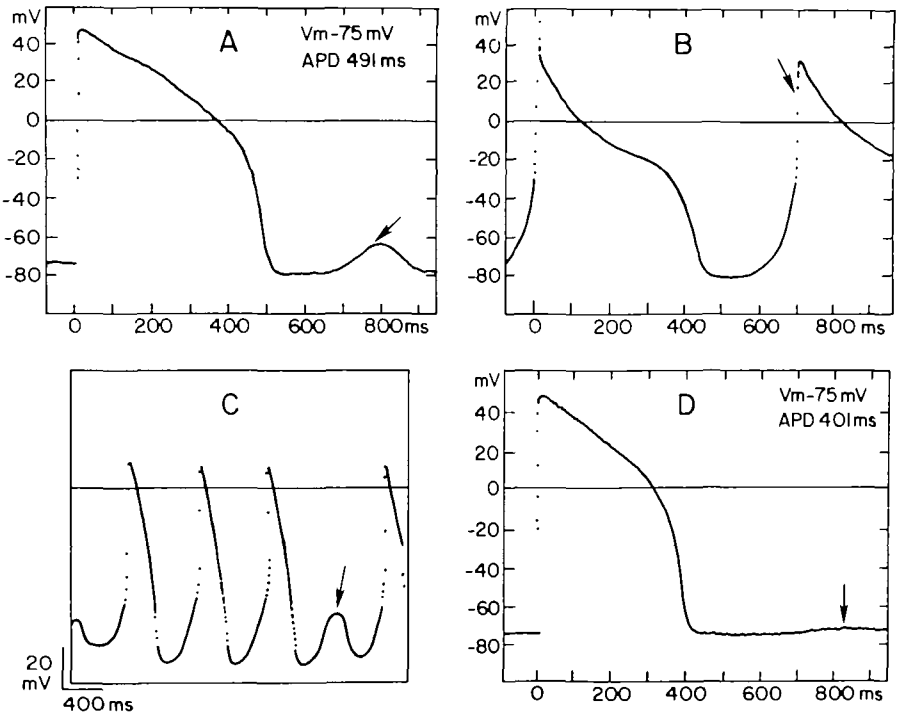

FIGURE 7. *Inhibition by adenosine (ADO) of the isoproterenol (ISO)-induced trigger automaticity in an isolated bovine ventricular myocyte. The resting membrane potential (V_m) and the action potential duration (APD) are shown in the right upper corner of panels A and D. Panel A: AP recorded in the presence of 10 nM ISO The arrow points to a delayed afterdepolarization Panels B and C. within 3 minutes of exposure to ISO, after a driven AP (first AP in Panel B), sustained rhythmic activity was triggered. The second AP in panel B is the first nondriven beat of the sustained rhythmic activity triggered by the preceding AP. In panel C (slower sweep speed), several spontaneous APs are shown. Note that the afterdepolarization indicated by the arrow (panel C) failed to reach threshold. Panel D: in the continued presence of ISO, addition of 50 μM ADO completely stopped the sustained rhythmic activity, reduced the AP duration, and abolished the delayed afterdepolarization (compare panels A and D). Not illustrated, washout of ADO (ISO still present) resulted in prompt resumption of the sustained rhythmic activity.*

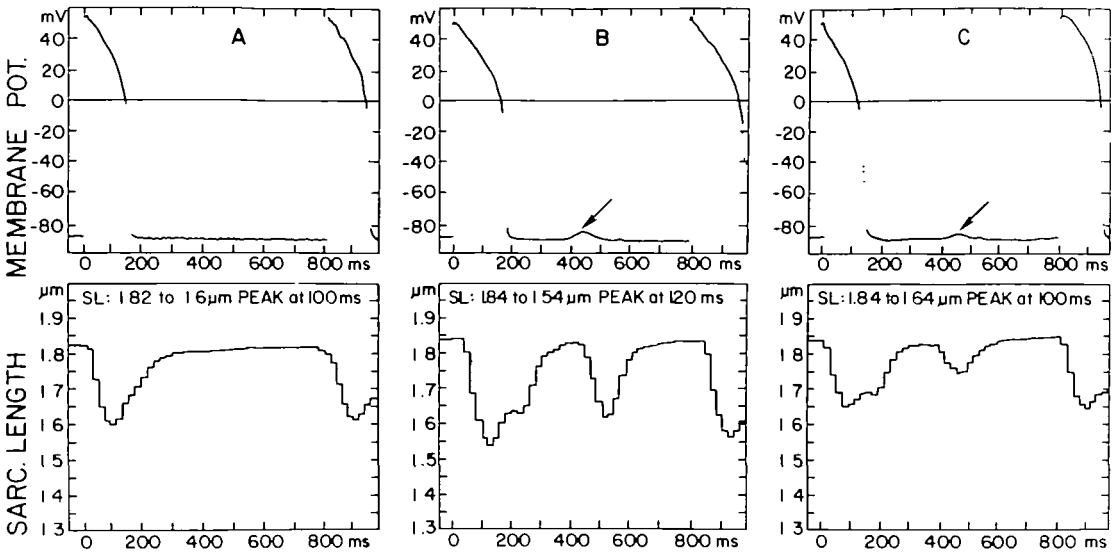

FIGURE 8. *Attenuation by adenosine (ADO) of the electrical and contractile effects of isoproterenol (ISO) in an isolated guinea pig ventricular myocyte. The action potentials (APs) are illustrated in the upper row (MEMBRANE POT) The bottom row (SARC LENGTH, SL) shows the unloaded shortening (downward deflection) and relengthening of the myocyte. Panel A: control AP accompanied by contraction of the myocyte Panel B: 10 nM ISO increased the extent of shortening and induced a delayed afterdepolarization and aftercontraction. Panel C: addition of 20 μM ADO in the continued presence of ISO reduced the extent of shortening, the amplitude of the delayed afterdepolarization and of the aftercontraction.*

present study, with its ability to antagonize the stimulatory effects of isoproterenol.

The positive inotropic effect of catecholamines in ventricular myocardium has been associated with increased levels of myocardial cAMP (Robison et al., 1965; Schumann et al., 1975; Dobson et al., 1976). Likewise, the shift of the action potential plateau toward the more positive potentials and the changes in action potential duration have also been suggested to be mediated by cAMP (Reuter, 1974; Beresewicz and Reuter, 1977). In fact, cAMP analogs and cAMP itself (applied internally) mimic the effects of externally applied catecholamines (Tsien, 1973; Reuter, 1974). In addition, the actions of catecholamines on the action potential have been shown to be related to their ability to increase Ca^{++} inward current (Vassort et al., 1969; Reuter and Scholz, 1977; Tsien, 1973).

Similar to the multicellular preparations, Ca-tolerant mammalian ventricular myocytes respond to adrenergic stimulation by increasing the cAMP content, by displacement of the plateau to more positive potentials, by prolongation of the action potential, by increasing contractility, and by increasing Ca^{++} inward current (Moustafa et al., 1976; Powell and Twist, 1976; Taniguchi et al., 1981; Powell et al., 1982; Isenberg and Klockner, 1981, 1982b; Isenberg, 1982). More recently, it was shown that cAMP injected into isolated guinea pig ventricular myocytes causes prolongation of the action potential and shifts the action potential plateau to more positive potentials (Trautwein et al., 1982). Thus, the documented electrophysiological effects of isoproterenol in the present study are in keeping with the above-mentioned reports from both multicellular and single cell preparations derived from ventricular myocardium. Furthermore, our results are also consistent with the observations that, in isolated guinea pig and cat papillary muscles, the contraction consists of two separated components, i.e., an early and a late component (Beresewicz and Reuter, 1977; Seibel et al., 1978). The two components of the contraction can be unmasked and better separated in the presence of noradrenaline or dibutyryl-cAMP, and in response to changes in stimulation rate (Beresewicz and Reuter, 1977; Seibel et al., 1978). In the isolated guinea pig ventricular myocytes, isoproterenol enhanced both components of the contraction; however, the enhancement of the early component was more distinct. This finding is similar to observations by Seibel et al., (1978) regarding the inotropic effects of dibutyryl-cAMP in the isolated guinea pig papillary muscle.

An additional manifestation of the actions of catecholamines is the enhancement and/or induction of afterdepolarizations that can result in sustained rhythmic activity (Wit and Cranefield, 1976, 1977; Lazzara et al., 1978). The characteristics (e.g., rate dependence) of the catecholamine-induced afterdepolarizations are similar to those of Purkinje fibers exposed to ouabain (Ferrier et al., 1973; Rosen et al., 1973). Our results show a remarkable similarity between the delayed afterdepolarization in the isolated ventricular myocytes and the ones from multicellular preparations. The isolated myocytes offer a unique advantage over the multicellular preparations to study this type of sustained rhythmic activity, since circus movement of excitation due to reentry can be completely ruled out in the case of the isolated cardiac myocytes. In regard to the underlying mechanism and the ionic basis of the depolarizing afterpotentials and aftercontractions, our re-

sults would be consistent with a secondary cytosolic calcium transient such as the type caused by adrenaline (Allen and Kurihara, 1980). As in the case of the other stimulatory actions of isoproterenol, its ability to increase afterdepolarizations and to induce triggered automaticity has been suggested to be mediated by cAMP (Lazzara et al., 1978). In fact, recently it was shown that, in isolated guinea pig ventricular myocytes, intracellular injection of cAMP increases the amplitude of the afterdepolarizations (Matsuda et al., 1982). It was also shown that intracellular Ca^{++} plays an important role in the genesis of the afterdepolarizations (Matsuda et al., 1982).

Regardless of the ultimate mechanism of the cardiac stimulatory action of catecholamines, the increase in cAMP levels and Ca^{++} influx seems to be the common feature. In the case of cardiac myocytes that develop afterdepolarizations and aftercontractions, the additional Ca^{++} influx caused by isoproterenol would lead to a state of Ca^{++} overload. In support of that, elevation of extracellular calcium (e.g., to 5.4 mM) also increased the amplitude of the afterdepolarizations and aftercontractions. This finding is consistent with the effects of extracellular calcium in the afterdepolarizations in Purkinje fibers, canine ventricular myocardium, and guinea pig ventricular myocytes (Ferrier and Moe, 1973; Lazzara et al., 1978; Matsuda et al., 1982).

Adenosine in concentrations ranging from 5 to 0.2 mM significantly attenuated the stimulatory actions of isoproterenol, i.e., both the electrophysiological and contractile effects. Interestingly, it has been previously reported that the inhibition by adenosine of the myocardial effects of catecholamines are related to its ability to attenuate the isoproterenol-induced increase in myocardial cAMP (Schrader et al., 1977; Dobson, 1978; Belardinelli et al., 1982). Adenosine (1 to 0.5 mM) itself does not affect the basal levels of cAMP (Schrader et al., 1977; Dobson, 1978; Belardinelli et al., 1982). Thus, provided the assumption that the effects of isoproterenol are due to increase in cAMP levels leading to an enhanced Ca^{++}-influx, the inhibitory actions of adenosine can be explained by its ability to antagonize the increased accumulation of cAMP produced by isoproterenol. In this context, it is anticipated that adenosine should reduce the cAMP-induced increase in Ca^{++} influx, and thus prevent its consequences, such as calcium overload. The present results, as well as the finding that adenosine attenuates the isoproterenol-induced necrosis in mice (Kulkarmi and Saraf, 1978), support such a hypothesis.

A final point relates to the similarities and differences between adenosine and calcium channel blockers such as verapamil and D-600. Both adenosine and calcium channel blockers may act by decreasing Ca^{++} influx into the cell but, adenosine merely antagonizes the additional influx of calcium caused by catecholamines. That is, adenosine would renormalize the excess cellular calcium load produced by catecholamines, whereas calcium channel blockers can lower the cellular calcium load to below basal levels.

In summary (1) the isolated myocytes respond to isoproterenol and adenosine despite the enzymatic dissociation, (2) in isolated ventricular myocytes, adenosine has no direct effect, but effectively antagonizes the effects of isoproterenol, and, (3) adenosine acts directly at the level of the cardiac myocyte, i.e., postjunctionally. The concentrations of adenosine required to attenuate the actions of isoproterenol are in the range of the concentrations of adenosine released by hypoxic and/or ischemic hearts (Thomas et al., 1976; Rubio et al., 1974). Thus, it is conceivable that endogenously released adenosine by cardiac cells, when O_2 availability is limited and/or demand is increased, may modulate the electrophysiological and contractile effects of exogenous catecholamines and/or increased sympathetic drive.

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INDEX TERMS: Action potential · Ca^{++}-tolerant myocytes · Adenosine · Isoproterenol · Isolated ventricular myocytes · Delayed afterdepolarization