Modification of the Cardiac Action Potential by Intracellular Injection of Adenosine Triphosphate and Related Substances in Guinea Pig Single Ventricular Cells

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SUMMARY. Effects of varying the intracellular adenosine triphosphate level on both the action potential and the membrane current were studied in single ventricular cells isolated from the guinea pig heart, using collagenase. Intracellular injection of adenosine triphosphate elevated the plateau potential level and prolonged the action potential duration. Similar results were obtained by injecting adenosine diphosphate, adenosine monophosphate, or creatine phosphate, i.e., substances considered to increase the intracellular concentration of adenosine triphosphate. In contrast, the action potential was depressed by procedures which could reduce the intracellular adenosine triphosphate level, such as an injection of creatine, superfusion of glucose-free Tyrode's solution containing 5.4 mM cyanide ion, or an injection of adenosine monophosphate into the cyanide-superfused cell. When the membrane current was recorded under the voltage clamp, it was found that the injection of adenosine triphosphate increased the amplitude of the slow inward current, whereas the superfusion of cyanide ion did not significantly decrease the slow inward current, although the action potential became considerably shorter. It was also found that the adenosine monophosphate injection decreased the amplitude of the net outward membrane current at the plateau level and increased it at around —40 mV, and thus intensified the N-shape of the isochronal 0.3-second current-voltage curve. The cyanide ion superfusion produced the opposite effect; in response to depolarizing clamp pulses more positive to the plateau level, the membrane current increased significantly with cyanide ion, but increased only slightly with adenosine triphosphate. These results suggest that intracellular adenosine triphosphate modifies the membrane currents at the plateau potential range, thus altering the action potential duration. (Circ Res 53: 131-139, 1983)

IT HAS long been known that the duration of the cardiac action potential shortens during hypoxia or in the presence of inhibitors of oxidative phosphorylation (for review, see Carmeliet, 1978). The primary effect of hypoxia on the cardiac muscle is a decrease of high energy phosphates such as ATP and creatine phosphate (CP) (McDonald and MacLeod, 1971), which results in a depression of active transport systems and in an increase of the intracellular concentration of free Na⁺, Ca²⁺, or H⁺ ions ([Na]i, [Ca]i, and [H]i) (Baumgarten et al., 1981). Therefore, a decrease of the slow inward current due to a decreased Ca²⁺ concentration gradient (Kohlhardt and Kübler, 1975; Kohlhardt et al., 1977), and/or due to intracellular acidification (Kohlhardt et al., 1976; Vogel and Spelakis, 1977; Kurachi, 1982), in addition to an increased background K current due to an increase of [Ca]i (Vluegels et al., 1976, 1980) have been considered as important factors for explaining the shortening of the action potential in hypoxia. However, McDonald and MacLeod (1973) found that the shortened action potential duration in hypoxia recovered if they raised the external glucose concentration to 50 mM, although the [Na]i still remained at a high level, compared with the normal condition. They proposed that the glycolytic ATP production of the muscle correlated to the plateau formation of the ventricular action potential. Vereecke et al. (1981) reported a similar effect on the action potential, as a result of stimulation of glycolysis in single myocytes of the guinea pig.

The experiments described in this paper were designed to clarify the relationship between [ATP] and the cardiac action potential or the membrane currents. We injected ATP and its related substances, i.e., ADP, AMP, creatine phosphate, creatine, or adenosine, into single guinea pig ventricular cells, or superfused the cell with cyanide (CN)-containing substrate-free solution. The results strongly suggested that the action potential duration is longer at higher [ATP], and shorter with lower [ATP]. We present voltage clamp experiments indicating that intracellular ATP regulates the membrane currents...
at the plateau potential range between −20 mV and +30 mV and thus influences the cardiac action potential duration.

Methods
Preparation of Single Ventricular Cells
Guinea pigs weighing 300–500 g were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg), and the chest was opened under artificial respiration (Taniguchi et al., 1981). The aorta was cannulated 5–6 mm above the heart, and the coronary artery was perfused continuously with normal Tyrode’s solution during which time, the heart was dissected and suspended on a Langendorff-type apparatus. After the blood was washed out, we perfused 50 ml of Ca²⁺-free Tyrode’s solution. The perfusate was then switched to Ca²⁺-free Tyrode’s solution containing 0.04% (wt/vol) collagenase (Sigma, type 1), which was recirculated for about 1 hour. Finally, 100 ml of a high K⁺ low Cl⁻ solution was perfused to wash out the enzyme. The heart was then removed from the perfusion apparatus and stored in the high K⁺ low Cl⁻ solution at 4°C for at least 1 hour before use. The solution was gassed with 100% O₂ and was perfused at 37°C under a hydrostatic pressure of 60 cm.

A small piece of tissue was taken from the heart, and cells were dispersed by teasing the tissue in the 1.8 mM Ca²⁺ Tyrode’s solution in the recording chamber. When the isolated cells settled at the bottom of the chamber, control Tyrode’s solution was perfused at a rate of 2–3 ml/min during the experiment. The perfusate was not gassed. Experiments were carried out at 36–37°C.

Solutions
The Tyrode’s solution contained (in mm), NaCl, 136.9; NaHCO₃, 11.9; KCl, 5.4; CaCl₂, 1.8; MgCl₂, 0.53; Na₂H₃PO₄, 0.33; glucose, 5.5; and before the experiment the pH was adjusted to 7.3–7.4 with 5 mM HEPES-NaOH buffer. The high K⁺ low Cl⁻ solution, first proposed by Isenberg and Klöckner (1980) as the high K⁺ substrate-enriched solution, was modified (in mm), taurine, 10; oxalic acid, 10; glutamic acid, 70; KCl, 25; KH₂PO₄, 10; glucose, 11; EGTA, 0.5; HEPES, 10 (pH 7.4 with KOH).

Since injection of the microelectrode into a cell often depolarized the cell membrane in 5.4 mM K⁺-Tyrode, most of the experiments were carried out in the Tyrode’s solution containing 10.8 mM K⁺ (control Tyrode’s solution), which facilitated stable recordings of the resting and action potentials. In preliminary experiments, we injected ATP into the isolated cells in both 5.4 mM K⁺ and 10.8 mM K⁺-Tyrode’s solution. The prolongation and elevation of the action potential plateau were observed at 5.4 mM K⁺ as well as in the 10.8 mM K⁺-Tyrode’s solution without any significant changes in the resting potential (see Results). CN⁻-Tyrode’s solution was prepared by omitting glucose and adding 5.4 mM KCN to the 5.4 mM K⁺-Tyrode’s solution and the pH was readjusted with HCl to 7.4.

A voltage-recording microelectrode was used for intracellular injection by filling with solutions described below. The resistance of the electrodes ranged between 30 and 100 MΩ. The action potential was elicited by passing a current pulse through the microelectrode (3 msec duration and 2–5 nA amplitude, 20/min).

Solutions for Injection
A 0.5 m solution of ATP [adenosine 5’-triphosphate potassium salt (Yamasa), 0.5 m ADP (adenosine 5’-di-phosphate potassium salt, Yamasa), 0.5 m AMP (adenosine 5’-monophosphate, Sigma), 5 m adenosine (Sigma) dissolved in 130 mM K⁺-aspartate, 0.5 m creatine phosphate (CP, Sigma), 87 mM creatine (Cr, Sigma) dissolved in 50 mM KCl and 150 mM EGTA were used for injection. The pH was adjusted to 7.2 with HEPES-KOH of 0.02–0.1 M. For the injection, a hydrostatic pressure of 98–294 kPa (1.0–3.0 kg/cm²) was applied on the recording electrode (for the injecting devices, see Trautwein et al., 1982). In preliminary experiments, injection of 150 mM K⁺-aspartate or KCl with a pressure below 3.0 kg/cm² for several seconds, did not cause any detectable change in the action potential configuration. The solutions for the injection were filtered with Millipore filters (pore size 0.05 μm).

With the present technique, we could not measure the exact volume of injection; neither could we estimate the final intracellular concentration of injected substances, because of the variable hydrostatic resistance of the electrode to pressure application. Thus, the effect was compared only qualitatively, but not quantitatively. We discarded the data when an increase in the cell volume was evident during the injection under the microscope.

Voltage Clamp
A two-microelectrode method was used for voltage-clamp experiments. The voltage-recording electrode was filled with 0.5 m ATP and 20 mHEPES (pH = 7.2), whereas the current-supplying electrode was filled with 3 m KCl. The resting potential recorded by these two electrodes did not differ from each other by more than 5 mV. Since the impalement of the second microelectrode depolarized most of the small cells, we selected relatively large cells (<150 × 50 μm) in order to record stable membrane currents with repeated clamp steps. As an indication for sufficient voltage homogeneity, Isenberg and Klöckner (1982) reported a difference of less than 0.4 mV between the two microelectrodes within a single bovine ventricular cell (200 × 45 μm), as long as applied current was smaller than 10 nA. As to the settling time of the voltage jump, the command step pulses of 10–30 mV depolarization from the holding potential of −40 mV was followed by the membrane potential with the time constant of about 0.4 msec. We could not evaluate the initial 1–2 msec after the onset of the voltage jump. The experiments were carried out only when the membrane current was stable and no transient oscillations recognized.

It was difficult to use Ca²⁺-blockers to separate ω, since the experiment was often interrupted by a sudden breakdown of cells during injection and/or in the presence of CN⁻. In preliminary experiments, however, we recorded currents on depolarization to 0 or +10 mV in the presence and absence of 2 μM D600. The difference current between the two traces was almost completely inactivated within 200 msec after the onset of the pulse. Therefore, we approximated the amplitude of ω by subtracting the current measured at 200–300 msec after the onset of the clamp pulse from the peak current, according to the “visual estimate” introduced by McDonald and Trautwein (1978), Isenberg and Klöckner (1980, 1982), and Osterrieder et al. (1982). All the data were recorded on magnetic tape for later analysis, using a Nicolet MED 80 computer.

Experimental Paradigm
Adenosine nucleotides, when injected intracellularly, may be incorporated into the following metabolic reactions.
The effects of injecting various adenine nucleotides, i.e., ATP, ADP, and AMP (0.5 mM), adenosine (5 mM), creatine phosphate (CP, 0.5 mM) and creatine (Cr 87 mM) on the action potential of isolated ventricular cells. The arrows indicate the direction of the response; the action potential was recorded 10–20 seconds after the start of injection with a pressure of 1 kg/cm². The initial upward deflection in the action potential is a stimulus artifact.

Figure 1. The effects of injecting various adenine nucleotides, i.e., ATP, ADP, and AMP (0.5 mM), adenosine (5 mM), creatine phosphate (CP, 0.5 mM) and creatine (Cr 87 mM) on the action potential of isolated ventricular cells. The arrows indicate the direction of the response; the action potential was recorded 10–20 seconds after the start of injection with a pressure of 1 kg/cm². The initial upward deflection in the action potential is a stimulus artifact.

Results

Effects of Varying [ATP], on the Ventricular Action Potential

Injection of ATP, ADP, or AMP into single ventricular cells caused prolongation of the action potential and elevated the plateau voltage, while the resting potential remained almost unchanged (Fig. 1, a–c). When the injection of ATP was continued, the rate of repolarization also increased from 2.4 to 4.5 V/sec in the average of three experiments. In most of the experiments, the injection was stopped when obvious prolongation of the action potential was detected. The effect persisted for 5–10 minutes after stopping the injection. In contrast to the effect of adenine nucleotides, injection of adenosine depressed the action potential (Fig. 1d). Recovery was observed within 4 minutes after the termination of the injection. Injection of CP also prolonged the action potential (Fig. 1e), whereas the injection of Cr shortened the action potential (Fig. 1f).

Table 1 summarizes the results of various injections. The resting membrane potentials did not appear to be affected in any case. A marked effect was observed on the duration of the action potential. Injection of ATP, ADP, AMP, or CP prolonged the action potential by up to 40–60% of the control, while injections of adenosine or Cr shortened it by about 40%. The amplitude measured 20 msec after the stimulus artifact, increased, accompanying the prolongation of the action potential in every experiment.

To decrease [ATP], the CN−-Tyrode’s solution containing 5.4 mM CN− was superfused. After several minutes of CN− superfusion, the action potential duration became shorter and the plateau disappeared within 10–20 minutes. Consistent changes in the resting potential were not observed. Neither transient depolarization after the action potential (Ferrier, 1977; Matsuda et al., 1982) nor shortening of the resting sarcomere length was observed during a 30-minute perfusion of CN−-Tyrode’s solution, indicating that [Ca]i did not increase up to the threshold of mechanical activation. Furthermore, injection of EGTA into CN-treated cells failed to recover the shortened action potential duration in any of the three experiments. After washing out CN−, the action potential recovered to the control level within 10 minutes.

These data may well indicate that the action potential duration is correlated to [ATP], because the prolongation of the action potential was observed under conditions which possibly raised [ATP], i.e., injections of ATP, ADP, AMP, and CP, whereas the opposite effect was elicited by injecting Cr or by superfusing CN− (see "Experimental Paradigm" in Methods, for CP; also Ventura-Clapier and Vassort, 1980).

Injections of ATP, ADP, and AMP into the CN−-Treated Cells

Besides being converted to ATP, injected ADP or AMP itself may influence the action potential. This possibility was tested by injecting ATP, ADP, or AMP into CN−-superfused cells in which the respiration and the glycolysis should be depressed so that the injected ADP and AMP should not be converted to ATP efficiently. The isolated cells were pretreated with CN−-Tyrode’s solution until the action potential duration became shorter than half of the control. When ATP was injected (Fig. 2b), the action potential immediately recovered and became even longer than the control. The amplitude also recovered with the injection (Fig. 2c). When the injection was stopped, the action potential diminished again (Fig. 2d). In eight experiments in which ATP was injected,
TABLE 1

Effects of Injecting Various Substances on the Ventricular Action Potential

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Maximum response</th>
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<tr>
<td></td>
<td>n</td>
<td>Rest (mV)</td>
</tr>
<tr>
<td>ATP</td>
<td>19</td>
<td>-60 ± 4</td>
</tr>
<tr>
<td>ADP</td>
<td>9</td>
<td>-64 ± 5</td>
</tr>
<tr>
<td>AMP</td>
<td>11</td>
<td>-60 ± 4</td>
</tr>
<tr>
<td>Adenosine</td>
<td>3</td>
<td>-61 ± 4</td>
</tr>
<tr>
<td>Cr</td>
<td>12</td>
<td>-58 ± 7</td>
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<tr>
<td></td>
<td>4</td>
<td>-60 ± 6</td>
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The resting potential (Rest), amplitude (Amp), and duration of the action potential in single guinea pig ventricular cells are shown. The effect of injections were reversible in most cases, and the maximum or the minimum value of the plateau duration was measured as the maximum response. The time to reach the maximum response after the onset of the injection is indicated. % Control: numerals indicate the percentage compared with those observed in normal Tyrode’s solution. The amplitude was measured 20 msec after the stimulus artifact, and the duration at 90% repolarization. Results are expressed as mean ± SD.

*P < 0.001; †P < 0.01.

Figure 2. Changes in the duration and the amplitude of the action potential during superfusion with CN⁻-Tyrode’s solution and subsequent ATP injection. During superfusion of a glucose-free, 5 mM CN⁻-solution, the action potential duration (b) was progressively shortened and the amplitude (c) measured 20 msec after the stimulus artifact decreased. At the top of the figure, the action potentials in the control (trace a) and in the presence of CN⁻ (trace b) are shown. The injection of ATP (indicated by the short bar above the time scale) with a pressure of 1 kg/cm² during CN⁻ superfusion immediately enhanced the action potential (the maximum response, trace c). After stopping ATP injection, the action potential shortened again in the CN⁻-Tyrode’s solution (trace d).

ADP was injected when the action potential was shortened to 20-50% of the control by the application of CN⁻ (Fig. 3A). In five of 10 experiments, the action potential recovered to the control level. In two experiments, it recovered to only 50% of control. In three experiments, no obvious recovery was detected in the presence of CN⁻, although after washing out CN⁻, the injection prolonged the action potential in the same cell.

In three cases of AMP injection in CN⁻-superfused cells, the duration and amplitude decreased further (Fig. 3B). This effect of AMP injection may be attributed to a decrease of [ATP], by reaction 2.

These findings support the view that, in the intact cells, the injections of ADP and AMP enhanced the action potential through the secondary increase of [ATP].

Effects of the ATP Injection on Membrane Currents

Effects of ATP on membrane currents were examined under the voltage clamp. To avoid the activation of the sodium current (iNa), the membrane potential was held at approximately -40 mV where the current was usually outward by 1–2 nA in the steady state. In the control solution, depolarizing clamp steps induced a transient flow of inward current followed by an almost steady current flow between -30 mV and +30 mV (see Fig. 4). At the potentials more positive to +30 mV, a time-dependent increase in the outward current was observed. In response to hyperpolarizing clamp steps, marked inward rectification was observed. After measuring the control current-voltage (I-V) relationship, the...
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Figure 3. Panel A: changes of the action potential duration induced by ADP injection in the control and in CN−-Tyrode's solution. In the experiment indicated by the open circles (O), the injection did not recover the action potential in the presence of CN−, whereas, in the other two experiments, full (Δ), or about 50% (●), recovery was induced by the injection. The time given in the figure indicates the interrupted period in each trace (minutes). Panel B: effects of AMP injection in the control Tyrode's solution and in CN−-Tyrode's solution, on the action potential duration. In insets a and c, the action potentials in the control Tyrode's solution and 12 seconds after the onset of the AMP injection, are superposed. In inset b, in CN−-Tyrode's solution, five successive records before, during, and after the injection were superimposed. Note the rate of shortening of the action potential was accelerated during the AMP injection. The solid line on the action potential trace indicates 0 mV and the dotted lines +50 and −50 mV. The time scale at the bottom indicates 200 msec.

The clamp was switched off and prolongation of the action potential was confirmed with injection of ATP (Fig. 4, top left panel). At the top right of Figure 4, the membrane current recorded after the injection (O) was compared with the control records (●), at depolarizations to +10 mV and +60 mV close to the reversal potential of the slow inward current. The ATP injection apparently decreased the net outward current at +10 mV and increased it at +60 mV. The time-dependent component of the outward current was not significantly changed at +60 mV.

In the initial I-V relationship, the negative peak at about +10 mV increased by 2.5 ± 1.0 nA after the ATP injection in six experiments. The amplitude of the current measured as the difference between the peak and the current level at 300 msec increased by a factor of 1.38 ± 0.13 during the ATP injection. This difference in current may not reflect the slow inward current exclusively, because the time-dependent potassium current, iK, may also be contributing. Between −30 mV and +30 mV, however, the activation of iK was not significant in most cells (see Figs. 4 and 6). To confirm whether the increase was at least partially due to iK, we calculated the difference between the currents recorded before, and after the injection shown in Figure 4A. The subtracted current decreased with a time course similar to that of iK (63% decayed within 43 msec), indicating that the change induced by the ATP injection is neither due to a time-independent current component (iK1), nor to iK, which shows a much longer time course.

The time course of inactivation of the net inward current in control and in ATP was plotted on the logarithmic scale by measuring the amplitude from the level at 300 msec (Fig. 5B). The time course was fitted well with two exponentials. The time constants were 6.3 and 41.3 msec in the control, and they prolonged to 13.5 and 78.6 msec after the ATP injection at +10 mV. The result in the control Ty-
rode's solution (1.8 mm Ca++) was very similar to that observed in the single bovine ventricular myocytes [Isenberg and Klockner, 1982], 6 msec and 40 msec for fast and slow components]. In seven experiments, the average time constants increased significantly with ATP, i.e., from 46.5 ± 7.5 to 74.4 ± 6.5 msec for the slow component and 5.3 ± 1.0 to 10.7 ± 2.5 msec for the fast component. Extrapolating the two exponentials to time zero revealed an increase in the amplitude of both components by the ATP injection.

Effects of the ATP injection on the time-dependent inward current. In panel A, the difference between the currents obtained before (O) and after (o) the injection of ATP was calculated. The difference current showed a similar time course to i_{\text{atp}}, suggesting that i_{\text{atp}} increased. In panel B, the time course of decay of the current measured between the peak and at the end of the pulse (dotted lines in panel A) was plotted on the logarithmic scale. The time course was fitted by two exponentials. The least squares fit on the later linear part gave the time constant of 41.3 msec for the control (O) and 78.6 msec after (o) the ATP injection. The currents were subtracted by the fitted line and plotted again on the same graph. The least squares fit to the fast components gave time constants of 6.3 msec (control) and 13.5 msec (after the injection). The data are from the same experiment as shown in Figure 4.

FIGURE 5. Effects of the ATP injection on the time-dependent inward current. In panel A, the difference between the currents obtained before (O) and after (o) the injection of ATP was calculated. The difference current showed a similar time course to i_{\text{atp}}, suggesting that i_{\text{atp}} increased. In panel B, the time course of decay of the current measured between the peak and at the end of the pulse (dotted lines in panel A) was plotted on the logarithmic scale. The time course was fitted by two exponentials. The least squares fit on the later linear part gave the time constant of 41.3 msec for the control (O) and 78.6 msec after (o) the ATP injection. The currents were subtracted by the fitted line and plotted again on the same graph. The least squares fit to the fast components gave time constants of 6.3 msec (control) and 13.5 msec (after the injection). The data are from the same experiment as shown in Figure 4.

Effects of CN⁻ on Membrane Currents

The depression of the action potential in the CN⁻-Tyrode's solution was also examined by the voltage clamp method. After the control currents had been recorded, the clamp was released to record action potentials with a stimulus frequency of 20/min and CN⁻-Tyrode's solution was perfused. When the duration of the action potential was halved after 4–6 minutes of perfusion of CN⁻ (the inset of Fig. 6), the membrane currents were recorded again. Figure 6 shows the currents in response to depolarizing clamp pulses to +10 mV and to +60 mV in the presence of CN⁻. The lower graphs show the initial and the 0.3-second I-V curves measured from the voltage clamp records. The peak amplitude of the net inward current was not significantly affected by CN⁻, but the time course of decay apparently was accelerated (Fig. 6, top left panel). However, we could not analyze whether the acceleration was due to faster inactivation of i_{\text{atp}}, or to an increase in time-dependent outward current components. The net outward current positive to −30 mV increased significantly and the negative slope region in the 0.3-second isochronal I-V curve almost disappeared (Fig. 6). It is evident that the major effect of CN⁻ is to increase the net outward current, which may underlie the shortening of the action potential.

Also, in the presence of CN⁻, the injection of ATP increased i_{\text{atp}} and almost restored the negative slope region of the 0.3-second I-V relation (triangles with dotted curves in Fig. 6), but the amplitude of the net outward current was not reduced by the ATP injection at the potentials positive to +50 mV. Therefore, the recovery of the negative slope induced by the ATP injection may be due to an increase in a non-inactivated fraction of i_{\text{atp}} and/or a decrease in an outward current component. Similar results were obtained in two other experiments. These findings again suggest that the formation of the negative slope depends on the intracellular level of ATP.
The upper limit of an increase in $[\text{ATP}]_i$ can be estimated. Potentials in the inset were recorded before and after the application curve was measured after 6 minutes of superfusion with CN$^-$. ATP where the symbols indicate the same as in the upper panels. The I-V current was not significantly affected by the ATP injection. The initial clamp pulse from the holding potential of $-40 \text{ mV}$. The traces in the lower row shows the effect of CN$^-$ (○) and that of the ATP injection (×) in the CN$^-$. Tyrode’s solution. The apparent negative slope conductance in the quasi-steady state disappeared with superfusion of CN$^-$, but it appeared again after the injection of ATP. The net outward current at $+60 \text{ mV}$ was increased by the CN$^-$-treatment, and this current was not significantly affected by the ATP injection. The initial and the 0.3-second I-V relationships were demonstrated in the graph, where the symbols indicate the same as in the upper panels. The I-V curve was measured after 6 minutes of superfusion with CN$^-$. ATP was injected for 3 seconds with a pressure of 1 kg/cm$^2$. The action potentials in the inset were recorded before and after the application of CN$^-$. The ATP-ADP ratio of 3.9 was reported in the isolated ventricular cell by Altschuld et al. (1981). Also, in perfused rat hearts, the approximate contents of ATP, ADP, and AMP were reported to be $25.6, 10.0$ and $1.3 \mu \text{mol/g}$ of wet weight, respectively (Grinwald et al., 1980). Assuming that the composition of adenosine nucleotides in isolated guinea pig cells was similar to that in the rat heart, and that CP injection converts all of intracellular ADP and AMP to ATP, then $[\text{ATP}]_i$ would increase by less than 1.5 times the control value. This suggests that a small change in $[\text{ATP}]_i$ could modify the cardiac action potential. In the control Tyrode’s solution, the injection of either ADP or AMP mimicked the effects of injecting ATP. This finding was explained by assuming that these substances were converted to ATP, which prolonged the action potential duration. This notion agrees well with the finding that isolated cells using collagenase retain their energy metabolism (Altschuld et al., 1981).

The increase in $i_o$ may be responsible for the increase in the amplitude measured 20 msec after the upstroke of the action potential during ATP injection (Table 1, Fig. 1). A noninactivated fraction of $i_o$ as well as the decrease of the membrane potassium conductance at the plateau potential range (Hall et al., 1963; Deck and Trautwein, 1964; $i_{K1}$ in Noble, 1962) may contribute to produce the negative slope region in the steady state I-V curve, and thus produce the plateau. In the mathematical model of the ventricular action potential, Beeler and Reuter (1977) succeeded in reconstructing the plateau by incorporating a relatively large noninactivated fraction of $i_{K1}$. In the present experiment, when the negative slope of the I-V curve was enhanced by the injection of ATP, the amplitude of the net outward current increased at around $-30 \text{ mV}$ and decreased at around $+10 \text{ mV}$. This finding may indicate that both of the noninactivated fractions of $i_o$ and the time-independent $K^+$ conductance increased simultaneously when [ATP] was increased. However, until now, definite experimental evidence indicating a significant amplitude of the noninactivated fraction of $i_o$ has been lacking. When $i_o$ was blocked by D-600 or verapamil in the isolated ventricular cell, the steady state I-V curve remained almost unchanged (Isenberg and Klöckner, 1980; Kurachi, 1982). The rectification of the $i_{K1}$ channel may be an important factor in determining the amplitude of the outward current at the plateau potential range. However, the effect of ATP on this channel still has not been studied. At present, there is no conclusive data indicating the exact nature of the negative slope region in the steady state I-V curve.

The effects of superfusing CN$^-$. Tyrode’s solution on the electrical activity of the ventricular cell is probably due to decreased [ATP]. The current records obtained in CN$^-$. Tyrode’s solution are very similar to those observed under the hypoxic condition in the cat ventricular papillary muscle (Vleugels

Discussion

The major findings of this study are that the injection of ATP, ADP, AMP, or CP prolonged the action potential duration, while the injection of Cr, superfusion of the CN$^-$. Tyrode’s solution, or the injection of AMP in CN$^-$.treated cells shortened the action potential. During the ATP injection, the amplitude of the net outward current at the plateau potential level decreased. In the CN$^-$.treated cells, the membrane current at the plateau level increased in the outward direction so that the negative slope region of the 0.3-second I-V curve almost disappeared. The amplitude of $i_o$ increased by the injection of ATP. Thus, the present study demonstrates a strong possibility that intracellular ATP changes the action potential duration through modification of the negative slope region in the steady state I-V curve.

With our present technique, the volume of the injected solution was difficult to measure. However, in the experiment in which CP was injected, the upper limit of an increase in [ATP] can be estimated.
et al., 1980), in which an increase in potassium background current was found. The increase in the amplitude of the time-dependent outward current in CN−-Tyrode’s solution (Fig. 6) may agree with an increase in the outward rectifying K+ current on application of DNP (Vereecke et al., 1981). The amplitude of i+ did not change significantly under the effect of DNP. Vleugels et al. (1976) found an increase in rate coefficient of K+ efflux during hypoxia in the chick embryonic heart. They attributed this phenomenon to an increase of [Ca]i (see also Isenberg, 1977). The depressant effect of free intracellular Ca++ on the plateau of the action potential is now established by injecting Ca++ or EGTA into the isolated single ventricular cell (Matsuda et al., 1982; Trautwein et al., 1982; Kurachi, 1982). The injection of EGTA failed to recover the action potential plateau. We cannot completely exclude the Ca++ chelating action of ATP (Fabiato and Fabiato, 1979) from mechanisms underlying the prolongation of the action potential on the ATP injection in the control Tyrode’s solution. However, increased [Ca]i may not be a major cause of depression of the action potential in the isolated cell superfused with CN− for 10–20 minutes.

The mechanism underlying the close relationship between [ATP]i and the membrane current is, however, still unclear. The finding that the membrane current was affected by variation of [ATP]i is at least ever, still unclear. The finding that the membrane current was affected by variation of [ATP]i is at least unclear. The finding that the membrane current was affected by variation of [ATP]i is at least unclear.

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