Reversal of the ATP-liganded State of ATP-sensitive K⁺ Channels by Adenylate Kinase Activity*

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The mechanism that promotes transition from the ATP- to the ADP-liganded state of ATP-sensitive K⁺ (Kₐ₅₇₆) channels and consequent channel opening in a cytosolic environment of high ATP concentration has yet to be understood. A mechanism examined here that could reverse the ATP-inhibited state is based on the action of adenylate kinase to catalyze phosphoryl transfer between ATP and AMP, resulting in transformation of ATP into ADP. In membrane patches excised from guinea pig cardiomyocytes, AMP alone did not affect channel behavior but increased the open probability of ATP-inhibited Kₐ₅₇₆ channels. This required MgCl₂ and a hydrolyzable form of ATP and was prevented by P¹·P²·di-adenosine-5'-pentaphosphate, an inhibitor of adenylate kinase. The single channel amplitude and kinetics of channel openings induced by the ADP-generating substrates of adenylate kinase, AMP and MgATP, were indistinguishable from the biophysical properties of the Kₐ₅₇₆ channel exhibited after addition of MgADP. In whole cell voltage-clamped cardiomyocytes, introduction of exogenous adenylate kinase along with millimolar MgATP and AMP induced a K⁺ current that was suppressed by a sulfonyleurea blocker of Kₐ₅₇₆ channels. Enriched sarcomemal membrane preparations were found to possess ATP-AMP phosphotransferase activity with properties attributable to an extramitochondrial isofrom of adenylate kinase. These results indicate that adenylate kinase is a naturally occurring component of sarcemmal membranes that could provide dynamic governance of Kₐ₅₇₆ channel opening through its phosphoryl transfer catalytic activity in the microenvironment of the channel.

ATP-sensitive K⁺ (Kₐ₅₇₆) channels are involved in signaling networks that transduce cellular metabolic events into membrane potential changes and have been implicated in glucose-induced insulin secretion in pancreatic β cells or ischemia-associated action potential shortening in heart muscle (1–5). Although the defining property of Kₐ₅₇₆ channels is their inhibition by intracellular ATP (5), which is readily demonstrable in excised membrane patches, as is the effect of ADP to reverse this ATP-inhibited state (1, 2, 6–9), the mechanism by which opening of this channel is governed in situ has not been elucidated. The major question still to be resolved is how transition from the ATP- to the ADP-liganded state is accomplished.

The common assumption that changes in the cytosolic concentrations of adenine nucleotides are the sole determinant of Kₐ₅₇₆ channel opening has been contested (2, 4, 6, 10–13). In cardiac cells, the ATP concentration (~5–10 mM) exceeds by over 100-fold the IC₅₀ value for Kₐ₅₇₆ channel closure (12, 13). This translates into a requirement for a change of two orders of magnitude in the intracellular ATP concentration, which is incompatible with cell viability, to achieve a mass action-induced change in the state of ATP-liganding and channel opening.

Considering that altered concentrations of cytosolic ATP and/or ADP are not readily detectable nor correlated with predictable changes in Kₐ₅₇₆ channel function, transition from the closed to the open state may be governed by a dynamic, rather than a static, property of adenine nucleotide metabolism. Although a component of the Kₐ₅₇₆ channel complex belongs to the ATP-binding cassette (ABC) superfamily of transporters (14–16), essentially all members of which possess ATPase activity, ATP-phosphohydrolase has not been reported as an inherent activity of the Kₐ₅₇₆ channel. Therefore, identifying another catalytic mechanism to accomplish transformation of ATP to ADP is in order. Adenylate kinase, which catalyzes the phosphorol transfer reaction between ATP and AMP resulting in the generation of ADP (17), conforms to these requirements. This process appears suitable since adenylate kinase in muscle has been proposed to operate as a transducing system coupling ATP signaling with its glycolytic generation. A key feature of this process is the conversion, at a regulatory site in glycolysis, of inhibitory ATP by the catalytic action of adenylate kinase using AMP as a phosphoryl acceptor (18). A second characteristic of this regulatory process is that it depends on the rate at which transformation occurs with no change in the cellular steady state levels of adenine nucleotide reactants (19). Also relevant is the observation that the insulin secretory response to glucose is correlated to the rate of adenylate kinase-catalyzed phosphoryl transfer (20).

Possible involvement of adenylate kinase in regulating the opening of cardiac sarcemmal Kₐ₅₇₆ channels was examined by determining if the ATP-inhibited state of these channels can be reversed when the second substrate required for adenylate kinase catalysis, AMP, is provided and whether adenylate kinase can be identified as a sarcemmal membrane-associated activity. Evidence is presented that adenylate kinase activity is detectable in cardiac sarcemla, a locale which permits it to assure an AMP-dependent increase in K⁺ current of ATP-
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inhibited channels. An adenylate kinase-promoted transition from the ATP- to the ADP-ligated state could provide a mechanism for $K_{\text{ATP}}$ channel opening even in a cytosolic environment of ATP concentrations sustained at levels that exceed the channel’s sensitivity toward ATP-induced closure.

EXPERIMENTAL PROCEDURES

Channel Recording—After cardiomyotonic, ventricular myocytes were dissociated from pentobarbital-anesthetized guinea pigs (21). In brief, the heart was perfused (37°C) with (in mM) 136.5 NaCl, 5.4 KCl, 1.8 CaCl$_2$, 0.53 MgCl$_2$, 5.5 glucose, 5.5 HEPES-NaOH (pH 7.4), then with the same solution without Ca$^{2+}$ in the absence and presence of collagenase (40 mg/100 ml), and finally with (in mM) 10 taurine, 10 oxalic acid, 25 KCl, 10 HEPES-KOH, 0.5 EGTA-KOH, 1.5 MgCl$_2$, and 10 HEPES-KOH (pH 7.3). A piece of ventricle was dissected and agitated in the recording chamber to isolate single myocytes, which were then superfused with (in mM) 140 KCl, 1 MgCl$_2$, 5 EGTA-KOH, and 5 HEPES-KOH (pH 7.3). The gigaohm seal patch clamp technique was employed in the inside-out configuration. Patch electrodes (5–7 MΩ), with fire-polished and coated tips, contained (in mM) 140 KCl, 1 MgCl$_2$, 5 EGTA-KOH, 5 HEPES-KOH (pH 7.3). Nicotinamides with ATP and ADP were added to the internal side of excised patches. Channel activity was measured, at a holding potential of −60 mV (22°C), using an amplifier (Axopatch-1C, Axon Instruments, Foster City, CA) and monitored on a high gain digital oscilloscope (VC-6025 Hitachi, Tokyo). Data were stored on tape using a PCM converter system (VR-10 Instrutech, New York), reproduced on a pass filtered at 1.5 kHz (3–2 dB) by a Bessel filter (Frequency Devices 902, Haverhill, MA), sampled at 6 kHz, and patched, in which $K_{\text{ATP}}$ channel activity was restored following washout of nucleotides, were analyzed using the BioQuest software. The threshold for judging the open state was set at half of the single channel amplitude. Channel activity, assessed by digitizing segments of current records and forming histograms of base line and open level data points, was expressed as $N_P/N$, (number of channels in the patch; $P_o$, probability of each channel to be open). Whole cell voltage-clamp recordings were performed in cardiomyocytes bathed in (in mM) 136.5 NaCl, 5.4 KCl, 1.8 CaCl$_2$, 0.53 MgCl$_2$, 5.5 glucose, and 5.5 HEPES-NaOH (pH 7.4) by means of the same patch-clamp amplifier using fire-polished pipettes (resistance, 3–5 MΩ) filled with (in mM) 140 KCl, 1 MgCl$_2$, 5 EGTA-KOH, 5 HEPES-KOH, and 5 ATP (pH 7.3). Whole cell currents, obtained in response to rectangular pulses from a holding potential of −50 mV to test potentials, were sampled on-line and analyzed off-line.

Isolation of Sarcolemmal Membranes—Sarcolemmal membrane fractions were isolated by differential centrifugation and sucrose gradient (22). In brief, guinea pig ventricles were placed in 0.6 M sucrose and 10 mM tris-base-HCl (pH 7.0, 0°C, supplemented with 1 μg/ml phenylmethylsulfonyl fluoride), and homogenized by Polytron. Homogenates were centrifuged at 12,000 $\times$ g (30 min at 4°C). The supernatant (diluted in 320 mM sucrose and 20 mM MOPS, pH 7.4, also supplemented with protease inhibitors) was then centrifuged at 95,000 $\times$ g (60 min 0°C). The pellet was resuspended, homogenized using a glass homogenizer and a teflon pestle, layered (at 0°C) over a 30% sucrose min 0°C). The pellet was resuspended, homogenized using a glass homogenizer and a teflon pestle, layered (at 0°C) over a 30% sucrose

Statistics—Data were expressed as mean ± S.E. Significance of differences between two means was determined by the Student’s t test, and a value of $p < 0.05$ was considered significant.

Materials—Chemicals were from Sigma.

RESULTS

AMP-dependent Increase in ATP-inhibited $K_{\text{ATP}}$ Channel Activity in Sarcolemmal Patches—Upon excision of a sarcolemmal membrane patch from a cardiac cell, multiple openings of $K_{\text{ATP}}$ channels appeared and were suppressed by 100 μM ATP (Fig. 1A). This ATP-inhibited $K_{\text{ATP}}$ channel activity was gradually reversed by 100 μM AMP (Fig. 1A). Consequently, $K_{\text{ATP}}$ channel activity, expressed by the slope of cumulative $N_P$, rose from 0.89 s$^{-1}$ (with ATP) to 1.59 s$^{-1}$ (with ATP plus AMP). $N_P$ (200 μM) increased the probability of $K_{\text{ATP}}$ channel opening inhibited by 1 mM ATP.

FIG. 1. AMP-induced increase in ATP-inhibited $K_{\text{ATP}}$ channel activity. A: upper trace, channel record with dotted line at zero current level; lower trace, open channel probability ($N_P$), corresponding to upper record, calculated over 2.5-s-long intervals. $A_1$, cumulative $N_P$, corresponding to segments 1 and 2 in A, calculated in ATP (circles, segment 1) and in ATP plus AMP (squares, segment 2). Results of linear regression presented as solid lines. $A_2$, summarized data (n = 8); before (open column) and after addition of 100 μM ATP in the absence (closed column) and presence (hatched column) of 100 μM AMP. Asterisk indicates significant difference between values obtained in the absence and presence of AMP. B, AMP (200 μM) increased the probability of $K_{\text{ATP}}$ channel opening inhibited by 1 mM ATP.

Adenylate Kinase Assays—Adenylate kinase activity was measured by enzyme-coupled spectrophotometry at 22–25°C (26). In the forward direction, ATP formation was coupled to NADH oxidation with phosphoenolpyruvate (1 mM), pyruvate kinase (3 units/ml), and lactic dehydrogenase (3 units/ml) in an assay mixture (1 ml) containing (in mM) 100 Tris-HCl (pH 7.5), 0.1 NADH, 1 ATP, 1 AMP, and sarcolemmal membranes or unfractionated homogenates (15 μg protein/ml). NADH oxidation was monitored at 340 nm using a spectrophotometer. In the reverse direction, ATP formation was coupled to NADP reduction with glucose (10 mM), hexokinase (2.5 units/ml) and glucose-6-phosphate dehydrogenase (1.25 units/ml) in an assay mixture (1 ml) containing (in mM) 100 Tris-HCl (pH 7.5), 100 KCl, 2 MgCl$_2$, 0.5 NADPH, 2 ADP, 0.5 NADP, and sarcolemmal membranes or unfractionated homogenates (15 μg protein/ml). NADP reduction was monitored at 340 nm. A molar absorbance value of 6.22 × 10$^3$ was used to convert absorbance to micromoles of product formed.

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100 μM AMP was added to the 100 μM ATP-inhibited channels (n = 8; Fig. 1A). Even when 1 mM ATP was used to inhibit K_{ATP} channel activity, addition of AMP (200 μM) enhanced the probability of K_{ATP} channel opening (Fig. 1B). AMP alone did not increase the probability of K_{ATP} channel opening without prior ATP-induced channel inhibition (Fig. 2). On average, the N_{Po} was 3.9 ± 0.8 prior to and 3.7 ± 0.7 following addition of 100 μM AMP (n = 4; p > 0.05).

Requirements for AMP-induced Opening of ATP-inhibited K_{ATP} Channels—The AMP-dependent increase in ATP-inhibited K_{ATP} channel opening required both the presence of MgCl₂ and a hydrolyzable form of ATP. Under Mg^{2+}-free conditions, K_{ATP} channel opening (N_{Po} at 3.6 ± 0.8) was suppressed by 100 μM ATP (N_{Po} at 0.3 ± 0.4), but this could not be reversed by 100 μM AMP (N_{Po} at 0.4 ± 0.5, p > 0.05; n = 4). Also, when K_{ATP} channel openings (N_{Po} at 4.1 ± 0.7) were inhibited by ATPP₆S (100 μM; N_{Po} at 0.4 ± 0.6), AMP (100 μM) no longer increased K_{ATP} channel activity (N_{Po} at 0.3 ± 0.5, p > 0.05; n = 4).

Sensitivity to an Inhibitor of Adenylate Kinase—A Mg^{2+}-dependent process in which AMP and ATP, but not ATPP₆S, appear to serve as reactants defines the requirements of ATP-AMP phosphotransfer catalyzed by adenylate kinase. Treatment of membrane patches with P₁, P₅-di-adenosine-5′-pentaphosphate (Ap₅A; 20 μM), a potent transition-state inhibitor of adenylate kinase-catalyzed phosphotransfer, prevented the increase in ATP-inhibited K_{ATP} channel opening dependent on AMP plus Mg^{2+} (Fig. 3). On average, the mean N_{Po} which was 3.9 ± 0.6 in the absence of nucleotides and 1.9 ± 0.5 following application of Ap₅A (20 μM), decreased to 0.2 ± 0.2 with addition of ATP (100 μM) but remained at 0.2 ± 0.3 after adding 100 μM AMP in the continuous presence of Ap₅A and ATP (n = 5).

Indistinguishable K_{ATP} Channel Behavior Evoked by the Substrates and Product of AMP-ATP Phosphotransfer—Since the product of adenylate kinase-catalyzed phosphotransfer between MgATP and AMP is ADP, single channel amplitude and kinetic properties of Mg-ADP-induced K_{ATP} channel openings were compared with AMP-induced opening of ATP-inhibited K_{ATP} channels. Analysis of amplitude histograms revealed that the single channel current amplitude of AMP-induced openings of ATP-inhibited K_{ATP} channels was 5.4 ± 0.3 pA at a holding potential of −60 mV (n = 4), a value virtually identical to that measured in 100 μM ADP (5.3 ± 0.4 pA; n = 4). Closed and open time distribution, recorded within a burst of AMP-induced openings of ATP-inhibited K_{ATP} channels, could be fit by a single exponential with time constants, τ_{closed} and τ_{open} of 0.43 and 2.83 ms, respectively (Fig. 4A), which were similar to time constants (τ_{closed} at 0.46, and τ_{open} at 2.18 ms) obtained for ADP-induced channel activity (Fig. 4B). Thus, the amplitude and kinetic properties of K_{ATP} channels induced by the substrates of adenylate kinase, AMP plus MgATP, were similar to openings induced by MgADP, the product of ATP-AMP phosphotransfer.

Adenylate Kinase-induced Glyburide-sensitive Outward Current—Introduction of purified adenylate kinase (200 units/ml), along with its two substrates ATP (5 mM) and AMP (1 mM), to the pipette solution perfusing the interior of voltage-clamped cardiomyocytes produced a pronounced outward current (Fig. 5). During the first minute after whole cell patch formation, no significant outward current could be measured (Fig. 5A and 5B, left panel), but after a lag of 5–10 min a prominent outward current developed (Fig. 5A and 5B, right panel). On average, the value of the outward current reached 0.92 ± 0.15 nA at a membrane potential of 0 mV (n = 3). This current was suppressed by 10 μM glyburide, a sulfonylurea blocker of K_{ATP} channels (Fig. 5A). When ATP (5 mM) alone was added to the pipette solution, the outward current at a membrane potential of 0 mV was essentially equal to zero (not illustrated; n = 19). In the presence of 5 mM ATP, addition of AMP (1 mM) to the pipette solution produced, within 15 min following patch formation, a small glyburide-sensitive outward current at positive potentials (0.23 ± 0.07 nA at +40 mV; n = 3; not illustrated). Thus, introduction of exogenous adenylate kinase to the ATP plus AMP-containing pipette solution promoted the induction
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FIG. 5. Adenylate kinase induced outward current in whole cell voltage-clamped cardiomyocytes. Adenylate kinase (200 units/ml; purified from rabbit muscle), ATP (5 mM), and AMP (1 mM) were added to the pipette solution. A, time course of development of adenylate kinase-induced current measured at the end of a 1000-ms-long depolarizing pulse applied from a holding potential of ~50 mV. Glyburide was added to the bathing solution. B, current records obtained at 1 min (left panel) and 10 min (right panel) following whole cell patch formation. Holding potential was ~50 mV. Rectangular 1000-ms-long pulses were applied (in 10-mV steps) from 125 to 0 mV. In this patch, with development of outward current, suppression of the inward component was observed.

of a glyburide-sensitive $K_{\text{ATP}}$ current.

Occurrence of Adenylate Kinase Activity in Enriched Sarcolemmal Fraction—Cardiac muscle membranes were isolated by a procedure that enriches the sarcolemmal fraction of disrupted heart muscle. The sarcolemma specific Na,K-ATPase activity was used as a reference standard to assess the degree of sarcolemmal membrane enrichment, and the presence of the mitochondrial marker, succinic dehydrogenase, was used to determine the magnitude of mitochondrial contamination. The procedure yielded a preparation of membranes 6-fold enriched in Na,K-ATPase activity (with a specific activity of 146 ± 2 nmol/min/mg protein in the membrane fraction versus 25 ± 2 nmol/min/mg protein in the unfractionated heart homogenate), and with reduced succinic dehydrogenase activity (from 125 ± 2 nmol/min/mg protein in the unfractionated homogenate to 75 ± 3 nmol/min/mg protein in the enriched sarcolemmal fraction). The ATP-AMP phosphotransferase activity in the sarcolemmal fraction was proportional to the concentration of protein (from 3.75 to 15 μg/ml; n = 16) present and exhibited the substrate requirements (Fig. 6A) and reversibility (Fig. 6B) characteristic of adenylate kinase. In the sarcolemma-enriched fraction, the specific activity of adenylate kinase, assayed in the direction of ADP formation, with 1 mM AMP and 1 mM ATP, was 30 ± 4 nmol/min/mg protein (n = 4; Fig. 6A). This represented a specific activity approximately one-third of that found in the unfractionated heart homogenate (i.e., 98 ± 8 nmol/min/mg protein, n = 4), which contained the vast majority of the total adenylate kinase activity as non-membrane associated. Raising the ionic strength of the buffer in which the sarcolemmal membrane fraction was suspended with potassium acetate (0.5–1.5 M, 90 min at 4°C), followed by pellet separation using centrifugation (100,000 x g for 1 h) and sucrose (0.5 M) gradient did not remove adenylate kinase activity, which was 121 ± 6 before (n = 4) and 143 ± 1 nmol/min/mg protein after such treatment (assayed in the formation of ATP production, n = 4; not illustrated). The persistence of ATP-AMP phosphotransferase as a membrane-associated activity after several serial dilutions, resuspensions, and rehomogenizations of the fractionation procedure indicated that it most probably represents a species of adenylate kinase tightly bound to cardiac membranes. The phosphotransferase activity was highly sensitive to the relatively specific inhibitor Ap5A (35 ± 1 and 85 ± 2% inhibition at 1 and 10 μM Ap5A, respectively; n = 7) and relatively insensitive to inhibition by the less potent analog P3,P4-di-adenosine-5'-tetraphosphate (14 ± 3% at 10 μM, n = 4), a characteristic of an extra-mito-

FIG. 6. Adenylate kinase activity in isolated sarcolemmal preparations. A, adenylate kinase activity was present only when both AMP and ATP (1 mM, left; n = 4) were provided to the enriched sarcolemmal membrane fraction but not in ATP (center; n = 4) or AMP (right; n = 4) alone. Adenylate kinase was measured by oxidation of NADH as ADP was produced from ATP and AMP. No ADP production was detected when sarcolemma was omitted (n = 4; not shown), which indicated absence of contamination with exogenous adenylate kinase activity. B, consistent with its bidirectional property, adenylate kinase activity was also measured when ADP (2 mM; closed circles) was provided to the sarcolemmal membrane fraction but not in the absence of ADP (open circles). Adenylate kinase was assayed by measuring formation of NADPH as ATP was produced from ADP. Adenylate kinase activity was essentially linear with time (tested up to 50 min; n = 4). The present study reveals that opening of ATP-inhibited sarcolemmal $K_{\text{ATP}}$ channels can be promoted by adenylate kinase activity within the microenvironment of the channel. This conclusion is supported by two lines of evidence: 1) the open probability of $K_{\text{ATP}}$ channels was up-regulated, in excised patches, by ADP-generating substrates of adenylate kinase and 2) adenylate kinase activity itself was found associated with sarcolemmal membranes where it catalyzed transformation of
ATP into ADP. These results suggest that reversal from the close to the open state of K\textsubscript{ATP} channels could be regulated in situ through localized phosphoryl transfer between ATP and AMP, resulting in transformation of ATP, the inhibitory ligand, into ADP, the channel activator.

**Substrates of Adenylate Kinase Enhance the Open Probability of K\textsubscript{ATP} Channels**—In the absence of ATP, AMP alone did not alter cardiac K\textsubscript{ATP} channel behavior, which is in accord with the sensitivity of these channels toward adenine nucleotides (1, 2, 4, 6, 16). But in the presence of ATP, AMP could increase the open probability of K\textsubscript{ATP} channels as previously reported in excised membrane patches from pancreatic β cells (7). It is unlikely that the AMP-dependent activation of K\textsubscript{ATP} channels resulted from AMP acting as a partial antagonist, in the presence of the full channel antagonist ATP, since adenine nucleotides inhibit cardiac K\textsubscript{ATP} channels irrespectively of whether Mg\textsuperscript{2+} is present or not (27), yet AMP required MgCl\textsubscript{2} to activate the opening of ATP-inhibited K\textsubscript{ATP} channels. Also, while poorly hydrolyzable ATP analogs do substitute for ATP as inhibitory ligands (1, 2, 4, 6, 8), replacement of ATP with ATP\textsubscript{S} prevented the activating effect of AMP on K\textsubscript{ATP} channels. The requirements for both Mg\textsuperscript{2+} as a cofactor and AMP and ATP as apparent substrates are indicative of a possible involvement of adenylate kinase, which catalyzes ATP\textrightarrow AMP phosphotransfer (17, 18), It is well established that when AMP and Mg\textsuperscript{2+} are provided, the adenylate kinase-catalyzed phosphotransfer converts ATP into ADP (17, 26). Such reaction could underlie the observed AMP-dependent opening of ATP-inhibited K\textsubscript{ATP} channels since the ATP-ligated state of K\textsubscript{ATP} channels is associated with channel opening either by antagonism of ATP inhibition or by direct channel activation (2, 6, 9, 28–31). In line with such assumption was also the finding that the biophysical properties of K\textsubscript{ATP} channel opening induced by the substrates of adenylate kinase, AMP plus ATP, were virtually indistinguishable from those induced by the product of catalysis, ADP. A role for adenylate kinase is further supported by the loss of AMP-induced opening of ATP-closed channels in patches treated with a selective inhibitor of adenylate kinase, A\textsubscript{P}\textsubscript{3}A, which has no effect on other AMP-dependent catalytic processes including the AMP-kinase activity (32). Furthermore, in clamped cardiomyocytes, introduction of exogenous adenylate kinase through the patch pipette, along with ATP and AMP, induced an outward current that was inhibited by glyburide, a sulfonylurea blocker of K\textsubscript{ATP} channels. Taken together, these observations may fulfill the established criteria for a catalytic process to regulate a specific ion channel behavior (33) and support the notion that adenylate kinase activity can modulate the opening of cardiac K\textsubscript{ATP} channels.

**Adenylate Kinase Activity Associated with Cardiac Sarcolemmal**—To account for the activation of K\textsubscript{ATP} channels by ADP-generating substrates in excised membrane patches, endogenous adenylate kinase activity must be in close proximity to the channels. Although adenylate kinase is among the most ubiquitous and diversely distributed of cellular enzyme activities (17, 28, 34), its occurrence as an associated protein of cardiac sarcolemmal membranes has never been reported. Thus far, the presence of adenylate kinase within a plasma membrane has only been described in erythrocytes and synaptosomes where it is tightly associated with the lipid bilayer (35–37). Evidence that this enzyme activity may be a constituent of cardiac sarcolemmal membranes was obtained, herein, in isolated sarcolemmal preparations that possessed ATP-AMP phosphotransferase activity with properties attributable to an extra-mitochondrial adenylate kinase isofrom most probably tightly bound to cardiac membranes (26, 34, 38–42). Close association of ion channel proteins with modulatory enzymes has been reported in the case of protein kinases (33) and may represent a mechanism by which cells achieve highly localized regulation of ion-channel function by otherwise ubiquitous biochemical processes.

**Adenylate Kinase-dependent Regulation of K\textsubscript{ATP} Channels**—It has been recently suggested that the microenvironment surrounding sarcolemmal K\textsubscript{ATP} channels may play a role in regulating the ATP-dependent channel gating (43). Herein, in an environment of millimolar concentrations of ATP, addition of AMP increased the probability of channel opening. This is in line with the reported property of adenylate kinase to catalyze rapid phosphorylation of AMP within restricted subcellular compartments of intact muscle cells with no measurable change in the overall cytosolic levels of AMP, ADP, or ATP (18, 19). An adenylate kinase-catalyzed transformation of ATP to ADP within a locale closely associated with sarcolemmal K\textsubscript{ATP} channels would provide a unique endogenous mechanism for channel opening even when cytosolic concentrations of ATP are at millimolar levels.

Adenylate kinase activity is an integral part of cellular energy phosphoryl transfer networks, which couple flux-generating (ATPases) and flux-responding (glycolysis and oxidative phosphorylation) processes and permit sequential transfer of phosphorys between nucleotides (18, 19). This is of importance since evidence for functional interactions between K\textsubscript{ATP} channels with both the flux-generating Na,K-ATPase and the fluxresponding glycolytic enzymes have been obtained within membrane patches (13, 44–46). Based on the current understanding of adenylate kinase activity (18, 20), it can be speculated that the switch between the ATP- and ADP-ligated states of K\textsubscript{ATP} channels could be governed by the rate at which AMP is generated at a remote signaling site and transferred by adenylate kinase phosphotransfer to the channel. Thus, adenylate kinase could be a determinant of the composition of adenine nucleotide species at the channel site and/or govern the duration of the ATP-ligated state of the channel by the rate it catalytically transforms ATP to ADP. In this regard, ADP could be viewed as a second messenger transducing adenylate kinase activity into K\textsubscript{ATP} channel behavior. Indeed, in addition to biophysical and biochemical data implicating this enzyme in the regulation of channel opening (present study; see also Refs. 7 and 20), evidence, obtained by analysis of mutated components of the K\textsubscript{ATP} channel protein complex, identifies domains within the channel complex responsible for the ADP-dependent channel activation (9).

At present, the precise relationship between K\textsubscript{ATP} channels and adenylate kinase is not known. One of the subunits of the K\textsubscript{ATP} channel complex is encoded by the sulfonylurea receptor, a member of the ABC superfamily (14–16). Similar to other ABC proteins, the sulfonylurea receptor contains two nucleotide-binding folds (14, 16). There is, however, limited homology between the respective nucleotide-binding domains in the sulfonylurea proteins and the adenylate kinase isofroms (47), which suggests that adenylate kinase activity may not be intrinsic to the K\textsubscript{ATP} channel complex per se. Yet, possible association between adenylate kinase activity and ABC proteins has been suggested. Recently, mutations in the adenylate kinase gene have been linked to loss of osmoprotection conferred by the ProU transporter, a member of the ABC family (48). Also, selective ligands of adenylate kinase (32) apparently can target certain members of the ABC superfamily, including the K\textsubscript{ATP} channel and the cystic fibrosis conductance regulator (49, 50).

Finally, up- and down-regulation of adenylate kinase activity correlate with the opening and closing of K\textsubscript{ATP} channels, respectively. In intact muscle cells, adenylate kinase-catalyzed
phosphoryl transfer is increased when oxidative phosphorylation or creatine kinase activity is impaired (18, 51), conditions known to enhance KATP channel opening (2, 13). Conversely, suppression of adenylate kinase activity accompanies glucose-induced insulin secretion in pancreatic β cells (20), which is associated with closure of KATP channels (1). Further understanding of the mechanisms regulating the activity of adenylate kinase may provide a previously unrecognized approach to regulate the KATP channel behavior.

Acknowledgments—We thank Dr. N. D. Goldberg and Dr. P. P. Dzeja for the impetus provided for this work.

REFERENCES
1. Ashcroft, S. J. H., and Ashcroft, F. M. (1990) Cell Signalling 2, 197–214
2. Nichols, C. G., and Lederer, W. J. (1991) Am. J. Physiol. 261, H1675–H1686
3. Lazarduski, M. (1994) C. Cardiovasc. Pharmacol. 24, S1–S5
4. Terzic, A., Jahangir, A., and Kurachi, Y. (1995) Am. J. Physiol. 268, C525–C545
5. Noma, A. (1983) Nature 305, 147–148
6. Findlay, I. (1994) J. Cardiovasc. Pharmacol. 24, S6–S81
7. Larson, O., Ammala, C., Bokvist, K., Fredholm, B., and Rorsman, P. (1993) J. Physiol. Lond. 461, 349–365
8. Terzic, A., Tung, R. T., and Kurachi, Y. (1994) Cardiovasc. Res. 28, 746–753
9. Nichols, C. G., Shyng, S. L., Nestorowicz, A., Glaser, B., Clement, J. P., Gonzalez, G., Aguilar-Bryan, L., Permutt, M. A., and Bryan, J. (1996) Science 272, 1785–1787
10. Ghosh, A., Ronner, P., Cheong, E., Khalid, P., and Matschinsky, F. M. (1991) J. Biol. Chem. 266, 22897–22892
11. Delmariy, P., Jonas, J. C., and Henquin, J. C. (1995) J. Clin. Invest. 96, 1738–1745
12. Decking, U. K., Reffelmann, T., Schrader, J., and Kammermeier, H. (1995) Am. J. Physiol. 269, H734–H742
13. Weiss, J. N., and Venketash, N. (1993) Cardiovasc. Drugs Ther. 7, 499–505
14. Aguilar-Bryan, L., Nichols, C. G., Wechsler, S., Clement, J., Boyd, A. E., Gonzalez, G., Herrera-Sosa, H., Nguy, K., Bryan, J., and Nelson, D. (1995) Science 268, 423–426
15. Inagaki, N., Goni, T., Clement, J. P., Namba, N., Inazawa, J., Gonzalez, G., Aguilar-Bryan, L., Seino, S., and Bryan, J. (1995) Science 270, 1166–1170
16. Inagaki, N., Goni, T., Clement, J. P., Wang, C. Z., Aguilar-Bryan, L., Bryan, J., and Seino, S. (1996) Neuron 16, 1011–1017
17. Noda, L. (1973) in The Enzymes, pp. 279–305, Academic Press, Inc., New York
18. Zeleznikar, R. J., Dzeja, P. P., and Goldberg, N. D. (1996) J. Biol. Chem. 270, 7311–7319
19. Zeleznikar, R. J., Heyman, R. A., Graeff, R. M., Walseth, T. F., Dowie, S. M., Butze, E. A., and Goldberg, N. D. (1990) J. Biol. Chem. 265, 300–311
20. Olson, L. K., Schroeder, W., Robertson, R. P., Goldberg, N. D., and Walseth, T. F. (1996) J. Biol. Chem. 271, 16544–16552
21. Terzic, A., Tung, R. T., Inanobe, A., Katada, T., and Kurachi, Y. (1994) Neuron 12, 885–893
22. Pitts, B. J. R. (1979) J. Biol. Chem. 254, 6232–6235
23. Forsburg, B., III (1982) J. Biol. Chem. 257, 12678–12684
24. Proverbio, F., Proverbio, T., and Marin, R. (1986) Biochim. Biophys. Acta 858, 202–205
25. King, T. (1967) Methods Enzymol. 10, 322–331
26. Huss, R. J., and Glaser, M. (1983) J. Biol. Chem. 258, 13370–13376
27. Findlay, I. (1988) Pflugers Arch. Eur. J. Physiol. 412, 37–41
28. Findlay, I. (1988) J. Membr. Biol. 101, 83–92
29. Tung, R. T., and Kurachi, Y. (1991) J. Physiol. (Lond.) 437, 239–256
30. Lederer, W. J., and Nichols, C. G. (1989) J. Physiol. (Lond.) 419, 193–211
31. Terzic, A., Findlay, I., Hosoya, Y., and Kurachi, Y. (1994) Neuron 12, 1049–1058
32. Lienhard, G. E., and Secemski, I. I. (1973) J. Biol. Chem. 248, 1121–1123
33. Esquerra, M., Wang, J., Foster, C. D., Adelman, J. P., North, R. A., and Levitan, I. B. (1994) Nature 369, 563–565
34. Savabi, F. (1994) Mol. Cell. Biochem. 134, 145–152
35. Heller, M., and Hanahan, D. J. (1972) Biochim. Biophys. Acta 255, 239–250
36. Nagy, A., Shuster, T., and Delgado-Escueta, A. (1989) J. Neurochem. 53, 1166–1172
37. Wong, P. C. L., and Cho, D. Y. H. (1989) Biochem. Int. 19, 881–888
38. Walker, E. J., and Dow, J. W. (1982) Biochem. J. 203, 361–369
39. Muller, C. W., and Schulz, G. E. (1992) J. Mol. Biol. 224, 159–177
40. Khoo, J. C., and Russell, P. J. (1972) Biochim. Biophys. Acta 268, 98–101
41. Brdiczka, D., Pette, D., Brunner, G., and Miller, F. (1988) Eur. J. Biochem. 5, 294–304
42. Kubo, S., and Noda, L. H. (1974) Eur. J. Biochem. 48, 325–331
43. Terzic, A., and Kurachi, Y. (1996) J. Physiol. (Lond.) 492, 385–404
44. Pribe, L., Friedrich, M., and Benndorf, K. (1996) J. Physiol. Lond. 492, 405–417
45. Ribault, B., Mirell, C., Johnson, D., and Levin, S. (1996) J. Gen. Physiol. 107, 231–241
46. Weiss, J. N., and Lamp, S. T. (1987) Science 238, 67–69
47. Suminami, Y., Kishi, F., Torigoe, T., and Nakazawa, A. (1988) J. Biochem. (Tokyo) 103, 611–617
48. Gutierrez, J. A., and Csonka, L. N. (1995) J. Bacteriol. 177, 390–400
49. Jovanovic, A., Alekseev, A. E., and Terzic, A. (1996) Naunyn-Schmiedeberg's Arch. Pharmacol. 353, 241–244
50. Quiton, P. M., and Reddy, M. M. (1992) Nature 360, 79–81
51. Dzeja, P. P., Zeleznikar, R. J., and Goldberg, N. D. (1996) J. Biol. Chem. 271, 12847–12851