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Effects of ADP upon the ATP-Sensitive K^+ Channel in Rat Ventricular Myocytes

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Summary. The effects of ADP upon the gating of ATP-sensitive K^+ channels from rat ventricular myocytes have been investigated by patch-clamp single-channel current recording experiments. ADP was applied to the internal surface of excised inside-out membrane patches and depending upon the experimental protocol and the concentration it was found that ADP could either inhibit or stimulate openings of ATP-sensitive K^+ channels. In the absence of inactivation, ATP-sensitive K^+ channels were inhibited by ADP in a dose-dependent manner. Partially inactivated channels, on the other hand, were stimulated by low (10 to 250 μM) and inhibited by high (>250 μM) concentrations of ADP. ATP-sensitive K^+ channels which were being inhibited by ATP (<1 mM) could be opened by the simultaneous application of ADP (50 μM to 1 mM). ADP had no effect upon channels inhibited by mM concentrations of ATP. The situation was further complicated when it was found that inhibition evoked by ADP was strongly attenuated by the presence of Mg^{2+} ions whilst channel stimulation, whether of partially inactivated channels or channels inhibited by ATP, required the presence of Mg^{2+} ions. The analog of ADP, ADPβS, always evoked inhibition of ATP-sensitive K^+ channels which was not affected by the presence or absence of Mg^{2+} ions.

Key Words cardiac muscle · K^+ channel · ATP · ADP · magnesium

Introduction

A potassium-selective ion channel which could be inhibited by adenosine triphosphate (ATP) applied to the internal surface of the membrane (K^+_{ATP} channel) was first described in cardiac muscle cells (Noma, 1983). The effects of other nucleotides such as ADP, GTP, UTP, ITP and CTP were similar to, though less effective than ATP (Noma, 1983; Kakei & Noma, 1984; Trube & Hescheler, 1984; Kakei, Noma & Shibasaki, 1985).

A very similar channel, i.e. one that was selective for potassium ions and inhibited by intracellular ATP, has been found in the insulin-secreting B-cells of mammalian islets of Langerhans (Cook & Hales, 1984). Studies of the B-cell K^+_{ATP} channel demonstrated, as for the cardiac muscle channel, a high sensitivity for ATP and lesser inhibition evoked by other nucleotides (Cook & Hales, 1984; Misler et al., 1986; Ribalet & Ciani, 1987). Other studies have revealed a more complex situation where K^+_{ATP} channels from insulin-secreting cells have been shown to be stimulated by the application of low (less than mM) concentrations of ADP, GTP and GDP (Dunne & Petersen, 1986*a*,*b*). ADP has also been found to reduce the inhibition of K^+_{ATP} channel activity evoked by ATP (Dunne & Petersen, 1986*a*; Kakei et al., 1986; Misler et al., 1986; Ribalet & Ciani, 1987). Thus it has been suggested that the activity of K^+_{ATP} channels which is observed in intact insulin-secreting cells and its modulation via the metabolism of glucose (Ashcroft, Harrison & Ashcroft, 1984; Findlay, Dunne & Petersen, 1985; Rorsman & Trube, 1985; Dunne et al., 1986; Misler et al., 1986; Ashcroft et al., 1987; Ribalet & Ciani, 1987) resulted from the balance of ATP/ADP within the cell rather than the concentration of ATP alone.

In view of these recent results the present study was undertaken to examine the effects that ADP has upon the K^+_{ATP} channel of cardiac myocytes. It has been found that whereas high concentrations of ADP always inhibited K^+_{ATP} channel activity, low concentrations (less than 0.5 mM) could stimulate K^+_{ATP} channels but only after their partial inactivation. It was also found that ADP modulated K^+_{ATP} channel inhibition evoked by ATP. This observation has profound implications for the physiological role of this K^+ conductance pathway in cardiac muscle.

Materials and Methods

The collagenase digestion of rat ventricles was performed according to established procedures (Powell, Terrar & Twist, 1980). Individual myocytes were obtained by cutting a small fragment of tissue from the ventricular myocardium and shaking it gently in a plastic petri dish which contained K^+-rich solution. Cells settled on to the base of the dish which was placed upon the

stage of an inverted microscope. All experiments were conducted at room temperature (20 to 22°C).

Single-channel currents were recorded with the methods of Hamill et al. (1981). The experiments described here used only the excised inside-out membrane patch configuration. The excised patch from which recording was being made was continuously perfused by a stream of solution from one of a series of piped outlets. The flow rate of the solutions was approximately 100 μl/min. Single-channel currents were recorded with a Dagan 8900 patch-clamp amplifier with a 10 giga-ohm feedback resistor and stored on tape (SE Labs Ltd. 7000). Experimental results were subsequently replayed onto a Gould 2400 pen-recorder.

The standard extracellular solution (Na^+-rich) contained (mM): 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES. The pH was adjusted to 7.4 using NaOH. The control intracellular solution (K^+-rich) contained (mM): 140 KCl, 10 glucose, 10 HEPES and 5 of either EDTA or EGTA; the pH was adjusted to 7.4 with KOH. This K^+-rich solution, with no added $MgCl_2$, will be referred to in the text as the 0 Mg^{2+} solution. When Mg^{2+} was required in K^+-rich solutions 1.4 mM $MgCl_2$ was added to K^+-rich solution which contained 5 mM EGTA which provided an estimated free concentration of 1 mM Mg^{2+} (Martell & Smith, 1974). This will be referred to in the text as K^+-rich solution containing 1 mM Mg^{2+}. No adjustments were made for the Mg^{2+}-binding properties of the adenosine nucleotides since at the concentrations used the nucleotides in solution would not have reduced the free concentration of Mg^{2+} sufficient to affect the behavior of the channels (Findlay, 1987*b*). When K^+-rich solution was used as an 'extracellular' medium EDTA or EGTA was not included and 2 mM $CaCl_2$ and 1 mM $MgCl_2$ were added. Nucleotides were added to K^+-rich solutions as required. Adenosine 5'-triphosphate (ATP, Sigma) was used as either Mg^{2+}- or Na^+-salts. Adenosine 5'-diphosphate (ADP, Sigma) was used as the K^+-salt. Adenosine 5'-0-(2-thiodiphosphate) (ADPβS, Boehringer) was used as the trilithium salt.

Results

K^+_{ATP} Channel Inhibition Evoked by ADP

Figure 1(*A*) illustrates a typical experiment which was performed with Na^+-rich solution in the patch pipette and K^+-rich solution bathing the internal surface of the membrane. In control K^+-rich solution up to seven single-channel current levels were observed. When 1 mM ADP was applied to the internal surface of the membrane patch K^+_{ATP} channel activity was strongly inhibited such that only brief openings of a single-channel current level were seen. Channel activity recovered completely when the ADP was washed away from the membrane. No channel openings were observed when 1 mM ATP was applied to the membrane. When 0.1 mM ATP was applied to the patch, openings of one to two single-channel current levels were visible, while the application of 0.1 mM ADP had only a weak inhibitory effect. In each of six preparations ADP evoked less inhibition of K^+_{ATP} channels than the same concentration of ATP. In other experiments 0.1 to 1.0 mM ADP inhibited K^+_{ATP} channel activity (four preparations), while 1 and 10 μM ADP had no effect (four preparations).

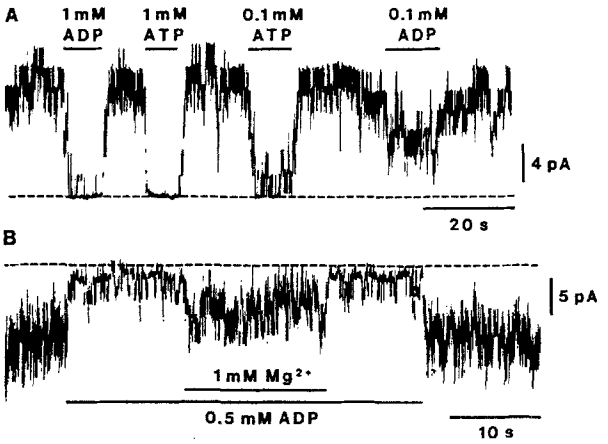

Fig. 1. Inhibition of K^+_{ATP} channels evoked by ADP and ATP. (*A*) A single-channel current record which was obtained with Na^+-rich solution bathing the external surface and K^+-rich solution without Mg^{2+} bathing the internal surface of an excised membrane patch. The membrane was voltage-clamped at 0 mV membrane potential. The dotted line represents the patch current level which was recorded when all channels were closed. Upward deflections of the current trace represent outwardly directed membrane currents, i.e., currents were passing from the interior to the exterior of the patch membrane. For the periods indicated by the bars above the current trace the K^+-rich solution which bathed the internal surface of the membrane also contained either 0.1 or 1.0 mM of ADP or ATP as indicated. (*B*) A single-channel current record which was obtained when an excised membrane patch was bathed upon both sides of the membrane with K^+-rich solution. The membrane was voltage-clamped at −40 mV membrane potential and currents which flowed through open K^+ channels passed from the exterior to the interior of the membrane and are shown as downward deflections of the current trace. The dotted line represents the patch current level which was recorded when all channels were closed. Under control conditions the K^+-rich solution contained no Mg^{2+}. The lower bar (0.5 mM ADP) indicates the period for which the patch was continuously perfused by control K^+-rich solution which contained 0.5 mM ADP. The upper bar (1 mM Mg^{2+}) indicates a period during which the K^+-rich solution with 0.5 mM ADP also contained 1.0 mM Mg^{2+}

Each of the experiments above were performed in 0 Mg^{2+} K^+-rich solution since it was found that the presence of Mg^{2+} greatly attenuated the inhibitory effect of ADP. The experiment shown in Fig. 1(*B*) was conducted with K^+-rich solution bathing both sides of the excised membrane and voltage-clamped at −40 mV membrane potential so that K^+ currents moved inwards through the open channels. This protocol was adopted to avoid block of K^+_{ATP} channel currents by internal Mg^{2+} ions (Findlay 1987*b*; Horie, Irisawa & Noma, 1987) though it resulted in the introduction of openings of the 'inward rectifier' K^+ channel to the experimental record

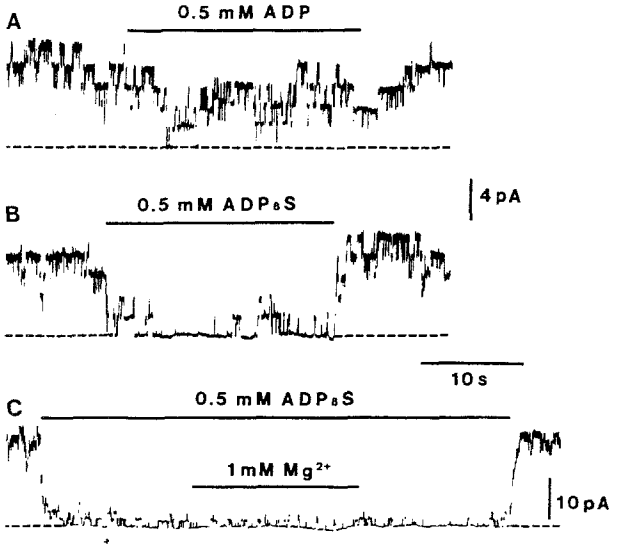

Fig. 2. Inhibition of K^+_{ATP} channels evoked by ADP and ADPβS. In this and all subsequent Figures the patch pipette contained Na^+-rich solution, the internal surface of the membrane was bathed in K^+-rich solution, the membrane was voltage-clamped at 0 mV membrane potential, outwardly directed open channel currents are shown as upward deflections of the current trace and the dotted lines represent the current level recorded from the patch when all channels were closed. This experimental protocol will hereafter be referred to as the Na^+/K^+ situation. (*A*) and (*B*) are two portions of a continuous record from one membrane patch. The K^+-rich bathing solution contained no Mg^{2+} throughout the experiment. For the periods indicated by the bars the patch was perfused with K^+-rich solutions which contained either 0.5 mM ADP (*A*) or 0.5 mM ADPβS (*B*). (*C*) A single-channel current trace recorded in a separate experiment. The control K^+-rich solution contained no Mg^{2+}. The upper bar (0.5 mM ADPβS) indicates the period for which the patch was continuously perfused by K^+-rich solutions which contained 0.5 mM ADPβS. The lower bar (1 mM Mg^{2+}) indicates when the K^+-rich solution with 0.5 mM ADPβS also contained 1.0 mM Mg^{2+}

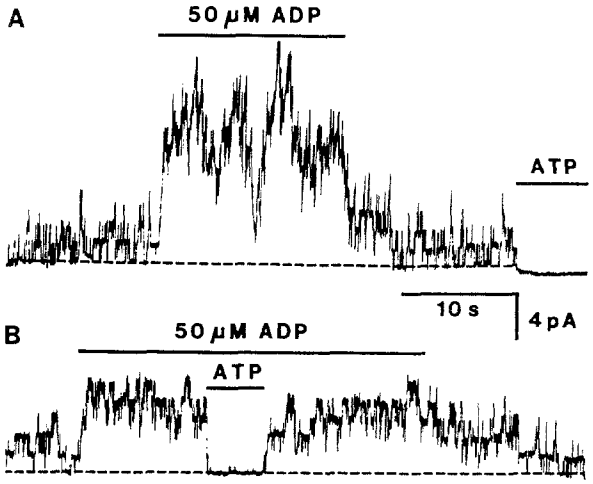

Fig. 3. Stimulation of K^+_{ATP} channels evoked by ADP. Single-channel current records were obtained from excised membrane patches in the Na^+/K^+ situation. (*A*) The bars above the current trace indicate periods for which 50 μM ADP and 2 mM ATP (ATP) were separately applied to the internal surface of the patch membrane. The K^+-rich solution contained 1 mM Mg^{2+} throughout the experiment. (*B*) The upper bar (50 μM ADP) indicates the period for which the patch membrane was continuously perfused with K^+-rich solution which contained 50 μM ADP. The lower bar (ATP) indicates a period for which the K^+-rich solution with 50 μM ADP also contained 2 mM ATP. The K^+-rich solution contained 1 mM Mg^{2+} throughout the experiment. (*A*) and (*B*) are traces which were recorded from separate experiments

(Trube & Hescheler, 1984). This type of channel was not affected by ADP. The application of 0.5 mM ADP to the internal surface of the membrane in 0 Mg^{2+} solution strongly inhibited K^+_{ATP} channel activity. When the patch was perfused with a K^+-rich solution which contained the same concentration of ADP but now also contained 1 mM Mg^{2+} much of the channel activity returned. The stronger inhibition was re-established when the Mg^{2+}-containing solution was washed away from the membrane. This particular protocol was repeated in three separate preparations with identical results in each case. In other experiments, which were conducted in the Na^+/K^+ situation, the inclusion of mM Mg^{2+}, which by itself blocks K^+_{ATP} channels (Findlay, 1987*b*), attenuated the inhibition of K^+_{ATP} channels which was evoked by 1.0 mM (five preparations), 0.5 mM (four preparations) or 0.25 mM (five preparations) ADP.

The nonhydrolyzable analog of ADP, ADPβS, also inhibited K^+_{ATP} channel activity. Figure 2 illustrates one of six experiments where ADPβS was compared with ADP at the same concentration. In each case ADPβS (Fig. 2*B*) was found to be a stronger inhibitor of K^+_{ATP} channel activity than ADP (Fig. 2*A*). Similar results were obtained in three other preparations where 0.1 and 0.05 mM ADPβS were compared with the same concentrations of ADP. In contrast to ADP (Fig. 1*B*), the inhibition of K^+_{ATP} channel activity evoked by ADPβS was not attenuated by the inclusion of mM Mg^{2+} (Fig. 2*C*). This result was obtained in each of four preparations.

K^+_{ATP} Channel Stimulation Evoked by ADP

Figure 3(*A*) illustrates that ADP, applied to the internal surface of an excised membrane patch, could stimulate opening of a K^+ channel. At the beginning of this record up to three single channels could be seen; 50 μM ADP stimulated the opening of up to 11 single-channel currents in this patch. Channel activity reverted to a low level when the ADP was washed away from the membrane. This residual activity was supressed by ATP. It has been shown that ADP will inhibit the same K^+ channel as ATP

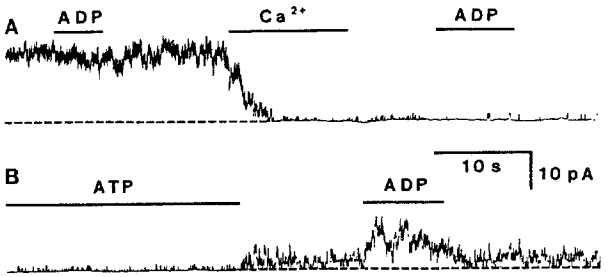

Fig. 4. K^+_{ATP} channels have to be inactivated before ADP will evoke stimulation. (*A*) and (*B*) form part of a continuous record which was obtained from one membrane patch in the Na^+/K^+ situation. Forty seconds of continuous record separates the two traces. 0.1 mM ADP (ADP), 0.1 mM Ca^{2+} (Ca^{2+}) or 2 mM ATP (ATP) were applied to the internal surface of the excised membrane for the periods indicated by the bars above the current record. The K^+-rich solution contained 1 mM Mg^{2+} throughout the experiment

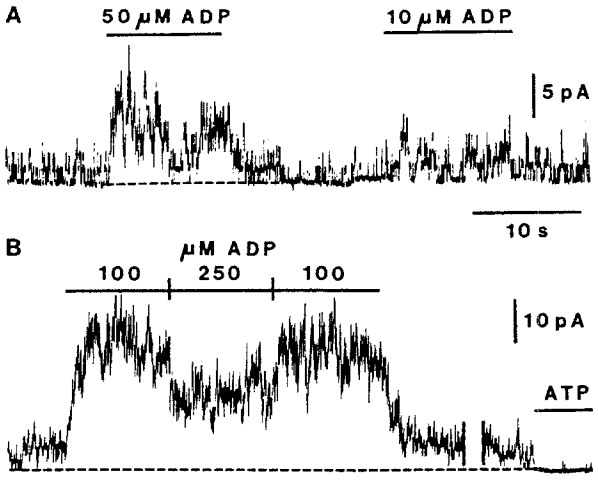

Fig. 5. Dose-dependent stimulation of K^+_{ATP} channels evoked by ADP. These single-channel current traces, obtained from excised membrane patches in the Na^+/K^+ situation, were recorded after the membranes had been exposed to the inactivation/reactivation protocol illustrated in Fig. 4 (*see* text for details). (*A*) For the periods indicated by the bars above the current trace the membrane was perfused with K^+-rich solution which contained first 50 then 10 μM ADP. (*B*) For the period indicated by the bar above the current trace the membrane was perfused with K^+-rich solutions which contained either 100 or 250 μM ADP as indicated on the Figure. Thirteen seconds of continuous record has been omitted from this trace (vertical bars). The patch was then perfused with K^+-rich solution which contained 2 mM ATP (ATP) as indicated by the bar. (*A*) and (*B*) were obtained from separate experiments, in both cases the K^+-rich solution contained 1 mM Mg^{2+} throughout the experiments

(Fig. 1). Does ADP also stimulate this channel? In Fig. 3(*B*) the application of 50 μM ADP increased channel activity from one to two current levels to more sustained openings of four to five channel current levels. The addition of 2 mM ATP in the continued presence of 50 μM ADP completely, and reversibly, inhibited channel activity in this patch. This experiment was repeated in five separate preparations with identical results in each case and thus it was concluded that the channel stimulated by ADP was the K^+_{ATP} channel.

There was clearly a difference between the circumstances where ADP evoked inhibition of K^+_{ATP} channel activity (Fig. 1) and where ADP evoked stimulation of K^+_{ATP} channel activity (Fig. 3). For ADP to evoke stimulation of K^+_{ATP} channel activity the run-down or inactivation of the channels was found to be a prerequisite.

K^+_{ATP} channel inactivation occurs in excised membrane patches which are exposed to bathing solutions which contain divalent cations (Findlay, 1987*b*). In the presence of mM Mg^{2+} inactivation is slow, with a time scale of minutes, and variable. This process can be accelerated by exposing the internal surface of the membrane to a high concentration of Ca^{2+}. K^+_{ATP} channel activity can then be recovered by exposing the membrane to MgATP. It was thus possible, in at least a reasonably controlled fashion, to create the conditions under which ADP stimulated K^+_{ATP} channels. The whole of this experimental protocol is shown in Fig. 4. The record commences approximately 20 sec after the membrane patch had been excised from a ventricular myocyte. The initial application of 0.1 mM ADP to the internal surface of the membrane was without effect. 0.1 mM Ca^{2+} was then applied to the internal surface of the membrane and caused the gradual but complete inactivation of the K^+_{ATP} channels which left occasional openings of another type of K^+ channel in the experimental record. This channel was not affected by intracellular nucleotides or calcium and it will not be considered further in this study. After the inactivation of the K^+_{ATP} channels the application of ADP again had no effect. The subsequent application of MgATP, which lasted for 30 sec, was without any apparent effect upon the experimental record until the ATP was washed away from the membrane and activity of some of the K^+_{ATP} channels was re-established. Now ADP markedly stimulated channel activity. In each of the experiments which will be described below the application of ADP before Ca^{2+}-stimulated inactivation and MgATP-evoked reactivation did not stimulate K^+_{ATP} channel activity.

With this experimental protocol, which will be referred to as the 'inactivation/reactivation protocol,' it was possible to demonstrate that ADP could stimulate K^+_{ATP} channel activity in a dose-dependent manner (Fig. 5). The single-channel current record shown in Fig. 5(*A*) commences with openings of one to two K^+_{ATP} channels. Fifty μM ADP increased this to a maximum of seven simultaneously open channels which was fully reversed upon removal of the

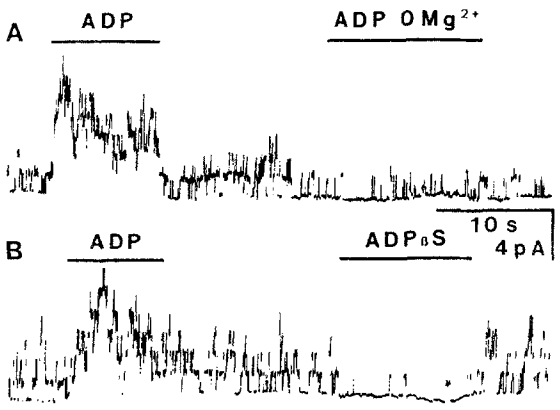

Fig. 6. ADP and Mg^{2+} are required to stimulate K^+_{ATP} channels (*A*) and (*B*) are single-channel current traces which were obtained from separate experiments. Both were recorded in the Na^+/K^+ situation after each had been exposed to the inactivation/reactivation protocol illustrated in Fig. 4 (*see* text for details). (*A*) The control K^+-rich solution contained 1 mM Mg^{2+}. 0.1 mM ADP (ADP) was first applied to the patch in control K^+-rich solution (bar). A second application of 0.1 mM ADP (ADP 0 Mg^{2+}) was made in K^+-rich solution which contained no Mg^{2+}. (*B*) The K^+-rich solution which bathed this patch contained 1 mM Mg^{2+} throughout the experiment. For the periods indicated by the bars above the current trace the K^+-rich solution also contained either 0.1 mM ADP (ADP) or 0.1 mM ADPβS (ADPβS)

ADP. 10 μM ADP only weakly stimulated K^+_{ATP} channel activity in the same membrane from one to two to a maximum of three single-channel current levels. This result was typical of experiments conducted in three separate preparations. Figure 5(*B*) illustrates an experiment where so many K^+_{ATP} channels were present in the excised membrane that a 'noise' record was obtained. That this corresponded to activity of K^+_{ATP} channels was confirmed at the end of this trace where the application of 2 mM ATP completely abolished the patch current. At the beginning of the record the patch current corresponded to that flowing through three to five open K^+_{ATP} channels. 100 μM ADP evoked a patch current which corresponded to approximately 20 simultaneously open K^+_{ATP} channels. When the concentration of ADP which bathed the internal surface of the membrane was increased from 100 to 250 μM the K^+_{ATP} channel current did not further increase but declined to a level which corresponded to 10 to 12 open channels. The reduction of the patch K^+_{ATP} current evoked by 250 μM ADP was fully reversed when the membrane was returned to solution which contained 100 μM ADP. It should be noted that although 250 μM ADP evoked less K^+_{ATP} channel current than 100 μM ADP, this was still considerably more than the patch showed in the absence of ADP. This result was confirmed in five separate experiments. In other experiments 0.1 mM (five preparations) and 0.25 mM (five preparations) ADP evoked stimulation of K^+_{ATP} channel activity whereas 0.5 mM ADP (four preparations) evoked their inhibition.

The stimulation of K^+_{ATP} channel activity by ADP was found to occur only in the presence of Mg^{2+} ions. Figure 6(*A*) illustrates an experiment where, after inactivation/reactivation, K^+_{ATP} channel activity was confined to opening of one to two single-channel current levels. The application of 0.1 mM ADP, in the presence of 1 mM Mg^{2+}, increased K^+_{ATP} channel activity to a maximum of six coincidentally open channels. The subsequent application of the same concentration of ADP but now in 0 Mg^{2+} K^+-rich solution resulted in a reduction in the frequency of K^+_{ATP} channel opening which recovered when the patch was returned to control solution (which contained 1 mM Mg^{2+}). This result was replicated in five experiments and also in five other experiments where 0.25 mM ADP stimulated K^+_{ATP} channel activity when it was applied in the presence of 1 mM Mg^{2+} but inhibited K^+_{ATP} channel activity when it was applied in the absence of Mg^{2+}. As noted previously 0.5 mM ADP evoked inhibition of K^+_{ATP} channels even after their inactivation/reactivation. This inhibition was enhanced when 0.5 mM ADP was applied in the absence of Mg^{2+} to the same patches (four preparations).

In a separate series of experiments membrane patches were excised and exposed to the inactivation/reactivation protocol in the absence of Mg^{2+} [except for the period of reactivation by MgATP (Findlay, 1987*b*)]. Subsequent applications of ADP in concentrations of 5 (five preparations), 10 (three preparations), 20 (three preparations), 40 (four preparations), 50 (five preparations) and 100 μM (four preparations) in the continued absence of Mg^{2+} failed to stimulate K^+_{ATP} channel activity. Instead, 40, 50 and 100 μM ADP each evoked mild inhibition of K^+_{ATP} channel activity in these experiments.

Figure 6(*B*) shows the result obtained from an experiment which used the inactivation/reactivation experimental protocol to examine whether the analog ADPβS could evoke K^+_{ATP} channel stimulation. In this and five other identical experiments even though ADPβS was applied in the presence of 1 mM Mg^{2+} it inhibited K^+_{ATP} channel activity whereas in the same patches the same concentration of ADP markedly stimulated the channels.

ATP-Evoked Inhibition of K^+_{ATP} Channels Is Modulated by ADP

ADP has been found to increase the activity of K^+_{ATP} channels which are being inhibited by ATP. In this series of experiments, which were not exposed to

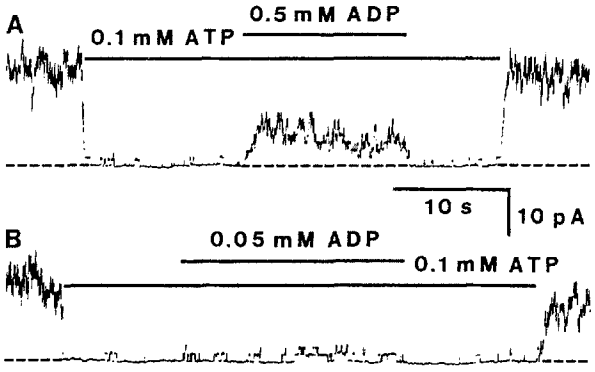

Fig. 7. The effect of ADP upon K^+_{ATP} channel inhibition evoked by ATP (*A*) and (*B*) form part of a continuous record from one membrane patch in the Na^+/K^+ situation. The K^+-rich solution contained 1 mM Mg^{2+} throughout the experiment. Fifty-five seconds of continuous recording separates the two traces. This patch had not been exposed to the inactivation/reactivation protocol. (*A*) The lower bar (0.1 mM ATP) indicates the period for which the patch membrane was continuously perfused with 0.1 mM ATP. The upper bar (0.5 mM ADP) indicates the period for which 0.5 mM ADP was included in the presence of 0.1 mM ATP. (*B*) This patch was again perfused with 0.1 mM ATP (lower bar) in the presence of which 0.05 mM ADP (upper bar) was also applied to the membrane

the inactivation/reactivation protocol, a concentration of 0.1 mM ATP was used to incompletely inhibit K^+_{ATP} channel activity so that any further modulation would be apparent. The experiment illustrated in Fig. 7(*A*) shows that 0.1 mM ATP reduced K^+_{ATP} channel activity from 12 to 15 open channels in the control solution to occasional openings of a single-channel current level. The addition of 0.5 mM ADP, in the continued presence of 0.1 mM ATP, markedly enhanced K^+_{ATP} channel activity such that a maximum of seven coincident channel openings were observed. This activity was lost when the patch was returned to solution which contained ATP alone and the initial level of channel activity was recovered when the patch returned to solution without ATP. Figure 7(*B*), taken from the same experiment, shows that 0.05 mM ADP in the presence of 0.1 mM ATP increased K^+_{ATP} channel activity from occasional openings of a single channel to more sustained openings of that level with occasional openings of a second channel current level. It is not shown but in the same experiment 0.1 mM ADP, in the presence of 0.1 mM ATP, evoked activity in two to three K^+_{ATP} channel current levels. Similar results to those shown in Fig. 7 were obtained in nine separate experiments.

In other experiments 1 mM ADP was applied to excised membrane patches in the presence of different concentrations of ATP. When 1 mM ADP was applied in the presence of 1 mM ATP it either had no effect or provoked only a very weak response of brief openings of K^+_{ATP} channels (11 preparations); in the presence of 0.5 mM ATP 1 mM ADP more strongly revoked the inhibition (six preparations) and in the presence of 0.1 mM ATP results equivalent to or stronger than that seen in Fig. 7(*A*) were obtained (five preparations).

K^+_{ATP} channel activity evoked by ADP in the presence of ATP was abolished in 0 Mg^{2+} solution. Figure 8(*A*) shows that of the 14 to 17 K^+_{ATP} channels which were visible when the patch was bathed in control K^+-rich solution only one to two and rarely a third level were observed after 0.1 mM ATP had been applied to the internal surface of the membrane. When 1 mM ADP was added, in the continued presence of 0.1 mM ATP, K^+_{ATP} channel activity was markedly stimulated and oscillated between six and 11 simultaneously open channels and briefly almost completely reversed the inhibition which had been caused by ATP. So far the experiment had been conducted in solutions each of which contained 1 mM Mg^{2+}. When 0 Mg^{2+} K^+-rich solution which contained 0.1 mM ATP and 1 mM ADP was applied to the patch channel activity was reduced to brief openings of a single-channel current level. This was noticeably less than that which had been observed when the patch had been exposed to 0.1 mM ATP alone. Channel activity fully recovered when the patch was returned to K^+ solution which contained ADP, ATP and Mg^{2+}. The effects of ADP with ATP and of ATP alone were also reversible. The experiment continued (Fig. 8*B*). The patch was perfused with K^+-rich solution which contained 1 mM Mg^{2+} and showed activity in approximately 15 K^+_{ATP} channels. The application of 0.1 mM ATP inhibited these K^+_{ATP} channels such that only one or occasionally two single-channel current levels were visible. The patch was then perfused with a 0 Mg^{2+} solution which contained 0.1 mM ATP. Two things were apparent. First, the amplitude of individual K^+_{ATP} channel openings was increased as their blockage by internal Mg^{2+} ions was removed (Findlay, 1987*b*; Horie et al., 1987). Second, there was a slight increase in the channel open probability. These effects were reversed when Mg^{2+} was readmitted to the ATP-containing solution. The results shown in Figs. 8(*A*) and (*B*) were replicated in six experiments. Other experiments were conducted entirely in the absence of Mg^{2+}. When 0.5 mM ADP was applied in the presence of either 0.1 or 0.5 mM ATP the inhibition of K^+_{ATP} channel activity evoked by ATP was enhanced by the simultaneous application of ADP (five preparations).

In contrast to ADP the analog ADPβS did not revoke channel inhibition when it was applied in the presence of ATP even though the experiments were

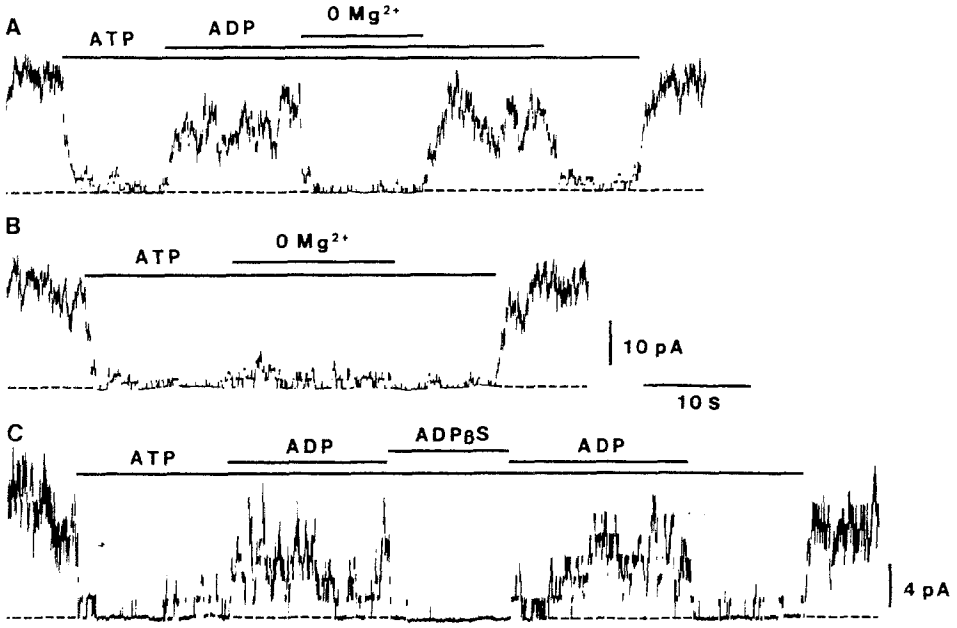

Fig. 8. ADP and Mg^{2+} are required to relieve ATP-evoked inhibition of K^+_{ATP} channels. These single-channel current traces were recorded in the Na^+/K^+ situation. (*A*) and (*B*) form part of a continuous record from one membrane patch. (*C*) is from a separate experiment. Neither patch had been exposed to the inactivation/reactivation protocol. (*A*) The lower bar (ATP) indicates the period for which the membrane was perfused with K^+-rich solution which contained 0.1 mM ATP. The middle bar (ADP) indicates the period for which the K^+-rich solution with 0.1 mM ATP also contained 1.0 mM ADP. The upper bar (0 Mg^{2+}) indicates a period for which the K^+-rich solution which contained both 0.1 mM ATP and 1.0 mM ADP contained no Mg^{2+}. At all other times the K^+-rich solutions contained 1 mM Mg^{2+}. (*B*) The lower bar (ATP) indicates the period for which the membrane was perfused with K^+-rich solution which contained 0.1 mM ATP. The upper bar (0 Mg^{2+}) indicates the period for which K^+-rich solution with 0.1 mM ATP contained no Mg^{2+}. At all other times K^+-rich solutions contained 1 mM Mg^{2+}. (*C*) In this experiment the K^+-rich solution which bathed the internal surface of this patch membrane contained 1 mM Mg^{2+} throughout. The lower bar (ATP) indicates the period for which the patch was perfused by K^+-rich solution which contained 0.1 mM ATP. The middle bars (ADP) indicate when the K^+-rich solution with 0.1 mM ATP also contained 0.5 mM ADP. The upper bar (ADPβS) indicates when the K^+-rich solution with 0.1 mM ATP contained 0.5 mM ADPβS

conducted in the presence of 1 mM Mg^{2+}. Figure 8(*C*) illustrates that in the continued presence of 0.1 mM ATP K^+_{ATP} channel activity was stimulated by ADP. But when ADPβS replaced ADP channel activity was lost. Channel activity returned when the patch was returned to solution which contained ADP instead of ADPβS. A different protocol (*not illustrated*) was also applied to each of the seven preparations of which Fig. 8(*C*) was a typical example, 0.5 mM ADPβS was applied to the patches in the continued presence of 0.1 mM ATP without an intervening exposure to ADP. In each case K^+_{ATP} channel inhibition evoked by ATP was enhanced by the addition of ADPβS.

The Effect of ADP Upon K^+_{ATP} Channels Blocked by Barium

It has been shown that ADP could stimulate K^+_{ATP} channels either when their activity had been reduced by inactivation or when they had been closed by ATP. Another method of at least apparently reducing the activity of these channels is to block them with a divalent cation applied to the internal surface of the membrane (Findlay, 1987*b*). Figure 9 illustrates an experiment which examined the effect

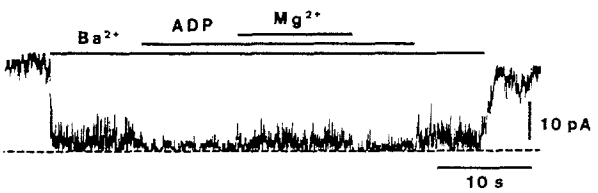

Fig. 9. Effect of ADP upon K^+_{ATP} channel activity which had been blocked by internal barium. A single-channel current trace recorded in the Na^+/K^+ situation, the control K^+-rich solution contained no Mg^{2+} and the patch had not been exposed to the inactivation/reactivation protocol. The lower bar (Ba^{2+}) indicates the period for which the K^+-rich solution which perfused the internal surface of the patch membrane contained 10 μM Ba^{2+}. The middle bar (ADP) indicates when the K^+-rich solution with 10 μM Ba^{2+} also contained 1.0 mM ADP. The upper bar (Mg^{2+}) indicates when K^+-rich solution with 10 μM Ba^{2+} and 1.0 mM ADP also contained 1.0 mM Mg^{2+}

of applying ADP to the membrane when K^+_{ATP} channel activity had been blocked by the application of 10 μM Ba^{2+}. In the absence of Mg^{2+} ions ADP inhibited K^+_{ATP} channel activity. In the presence of mM Mg^{2+}, ADP had little effect. In each of six experiments ADP did not relieve the block of K^+_{ATP} channels which had been evoked by the application of Ba^{2+}.

Discussion

The results of this study show that ADP can provoke different responses from K^+_{ATP} channels which depend upon the state of the channels, the concentration of the ligand and the presence or absence of Mg^{2+} ions. It is possible that some of these differences might be resolved if one considers which form of the ligand affected the channel. This study confirms that ADP can inhibit the activity of the K^+_{ATP} channel in cardiac muscle cells, though this inhibition is less than that evoked by similar concentrations of ATP (Noma, 1983; Kakei & Noma, 1984; Trube & Hescheler, 1984; Kakei et al., 1985). What is shown here for the first time is that the inhibition evoked by ADP is reduced in the presence of Mg^{2+} ions. It has recently been shown for the K^+_{ATP} channel of insulin-secreting cells that the free acid of ATP (ATP^{4-}) inhibited the channel and ATP complexed with divalent cations and in particular ATP·Mg had no effect (Ashcroft & Kakei, 1987; Dunne, Illot & Petersen, 1987). If it was only the free acid of ADP which evoked closure of the K^+_{ATP} channel in this study it is clear that the inclusion of mM Mg^{2+} should attenuate channel inhibition, since in a physiological solution without Mg^{2+}, ADP would exist predominantly as the free acid ADP^{4-}; in a solution with Mg^{2+} the concentration of ADP^{4-} would be reduced by Mg^{2+} binding.

This study shows, for the first time, that ADP can stimulate the activity of the K^+_{ATP} channel in a cardiac muscle cell. Dunne and Petersen (1986*a*) have shown similar results for the K^+_{ATP} channel of an insulin-secreting cell line. In both cases the 'run-down' or 'inactivation' of K^+_{ATP} channels was required before ADP would evoke stimulation. The accumulated evidence concerning the inactivation and reactivation of K^+_{ATP} channels suggests that divalent cation-dependent dephosphorylation and Mg·ATP-fuelled phosphorylation of the channel protein underlays these processes (Findlay & Dunne, 1986; Misler et al., 1986; Findlay, 1987*a*,*b*; Ohno-Shosaku, Zunkler & Trube, 1987). How then does ADP increase the open probability of the K^+_{ATP} channel and why is it only effective against partially activated channels?

Clearly, when the channels are fully active no further increase is possible. Similarly, when the channels are fully inactivated they cannot be opened, unless ADP revoked that inactivation. This was inferred by Dunne and Petersen (1986*a*) when the nonhydrolyzable analog ADPβS did not evoke stimulation. Stimulation which was evoked by GTP and GDP and their analogs GppNHp, GTPγS and GDPβS was interpreted as a cooperative effort between the guanosine-base nucleotides and ATP in the maintenance of channel activity (Dunne & Petersen, 1986*b*). The fact that none of these responses occurred in the absence of Mg^{2+} ions (Findlay, 1987*a*) and that each was quantitatively modulated according to the state of inactivation of the channels (Dunne & Petersen, 1986*a*,*b*; Findlay, 1987*a*) led to an alternative suggestion that there may be a nucleotide site which stimulated channel opening (Findlay, 1987*a*). This suggestion is supported by the observation that ADP would not stimulate channels that were completely inactivated (Fig. 4). That Mg^{2+} was a necessary cojoint with ADP and the other nucleotides and analogs for channel stimulation (Findlay, 1987*a* and this study) raises the interesting alternatives that either Mg^{2+} in some fashion makes available the stimulatory site or that it may be selective for the Mg^{2+}-complexed forms of the nucleotides. If the latter were true and ADPβS did not significantly bind Mg^{2+} it would explain why it, alone, fails to evoke stimulation (Dunne & Petersen, 1986*a*; Findlay, 1987*a*). It is unfortunate that a quantitative dose-response curve for stimulation evoked by ADP is not available at the present time. But inactivated channels are in an unstable state which changes with time and also varies greatly from patch to patch. Thus, quantitatively, the response to ADP (and to other nucleotides) varies not only between experiments but even within the same experiment (Dunne & Petersen, 1986*a*,*b*; Findlay, 1987*a*). Qualtitatively it is clear that at low concentrations in the presence of Mg^{2+}, ADP can stimulate channel opening; as the concentration of ADP increases, perhaps because of the proportionate increase in the concentration of ADP^{4-}, stimulation declines and eventually inhibition dominates the channels' response.

In both cardiac muscle and insulin-secreting cells, K^+_{ATP} channels have a high affinity for ATP (Noma, 1983; Cook & Hales, 1984; Kakei et al., 1985; Rorsman & Trube, 1985). It is clear in both types of tissue that inhibition of the K^+_{ATP} channel evoked by ATP is markedly attenuated by the simultaneous presence of ADP (Dunne & Petersen, 1986*a*; Kakei et al., 1986; Misler et al., 1986; Findlay, 1987*a*; Ribalet & Ciani, 1987). It has been suggested that ADP, a weak agonist, competed with

ATP, a strong agonist, for the nucleotide binding site which evokes closure of the channel (Misler et al., 1986; Ribalet & Ciani, 1987). However, the results which show that ADP can stimulate K^+_{ATP} channels in the absence of ATP (Dunne & Petersen, 1986*a*; Findlay, 1987*a* and this study) and the fact that it has yet to be shown that ADP can revoke ATP-inhibition in the absence of Mg^{2+} indicate that the situation may be more complex. The extent to which ADP displaces ATP from the inhibitory binding site (without itself evoking channel closure) and/or the influence of ADP · Mg binding to a separate stimulatory site determines the behavior of a channel which is exposed to a mixture of nucleotides and their 'sub-species' in solution must wait for a full quantitative analysis which takes account of all these possible factors.

Whatever is the mechanism which is finally determined to be responsible for ADP causing a rightward shift of the dose-response curve for inhibition evoked by ATP, it is clear that the existence of this phenomenon has profound implications for the physiological role of the K^+_{ATP} channel. In insulin-secreting cells it appears to be clear that the K^+_{ATP} channel has a role in maintaining a high resting membrane potential which is then reduced by the metabolic inhibition of the K^+_{ATP} channels (Ashcroft et al., 1984; Rorsman & Trube, 1985; Dunne et al., 1986; Misler et al., 1986; Ribalet & Ciani, 1987). In cardiac muscle cells K^+_{ATP} channels have not been observed in resting isolated myocytes (Noma, 1983; Trube & Hescheler, 1984) though to what extent these cells accurately reflect the metabolic situation of a myocyte in a working heart is not known. What is known is that when the metabolism of intact hearts or isolated myocytes is compromised either by the interruption of the supply of nutrients ('ischemia') or by the application of metabolic poisons the duration of the cardiac action potential is reduced and this is associated with an increase in an outward K^+ current (Isenberg et al., 1983; Hasin, Doorey & Barry, 1984). The observation, first that a K^+ channel could be regulated via a product of cellular metabolism, i.e., ATP, then that K^+_{ATP} channels were active in myocytes which had been poisoned (Noma, 1983; Trube & Hescheler, 1984), and that during 'ischemia' cellular ATP content plummets (Hasin et al., 1984; Humphrey, Holliss & Seelye, 1984; Bukoski & Sparks, 1986; Sellevold, Jynge & Aarstad, 1986), pointed to the K^+_{ATP} channel as underlying the 'ischemic K^+ current.' The precise role of this current is not clear. It would certainly reduce the work done and thus the rate of metabolism of individual myocytes by reducing the duration of the action potential and Ca^{2+} influx. It may also prevent the generation of extraneous spike potentials by holding the membrane potential at values close to the equilibrium potential for K^+. The effectiveness of this cardio-protective role for the K^+_{ATP} channel would be enhanced by ADP since K^+_{ATP} channel activity would occur in the presence of much higher concentrations of ATP than had previously been thought.

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