Ligand-insensitive State of Cardiac ATP-sensitive K⁺ Channels

Basis for Channel Opening

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ABSTRACT The mechanism by which ATP-sensitive K⁺ (K_ATP) channels open in the presence of inhibitory concentrations of ATP remains unknown. Herein, using a four-state kinetic model, we found that the nucleotide diphosphate UDP directed cardiac K_ATP channels to operate within intraburst transitions. These transitions are not targeted by ATP, nor the structurally unrelated sulfonylurea glyburide, which inhibit channel opening by acting on interburst transitions. Therefore, the channel remained insensitive to ATP and glyburide in the presence of UDP. “Rundown” of channel activity decreased the efficacy with which UDP could direct and maintain the channel to operate within intraburst transitions. Under this condition, the channel was sensitive to inhibition by ATP and glyburide despite the presence of UDP. This behavior of the K_ATP channel could be accounted for by an allosteric model of ligand-channel interaction. Thus, the response of cardiac K_ATP channels towards inhibitory ligands is determined by the relative lifetime the channel spends in a ligand-sensitive versus -insensitive state. Interconversion between these two conformational states represents a novel basis for K_ATP channel opening in the presence of inhibitory concentrations of ATP in a cardiac cell.

KEY WORDS: K_ATP channel • nucleotide diphosphate • kinetic model • allosteric model • sulfonylurea

INTRODUCTION

ATP-sensitive K⁺ (K_ATP) channels transduce cellular metabolic events into membrane potential changes (Ashcroft and Ashcroft, 1990; Lazdunski, 1994; Seino et al., 1996; Bryan and Aguilar-Bryan, 1997), which in heart muscle lead to shortening of action potential duration during ischemia (Nichols and Lederer, 1991; Findlay, 1994; Terzic et al., 1995). The defining property of K_ATP channels is their inhibition by intracellular ATP (Noma, 1983). In cardiomyocytes, however, the ATP concentration (∼5–10 mM) exceeds by >100-fold the IC₅₀ value for K_ATP channel closure. Thus, a change of two orders of magnitude in the ATP concentration would be required for channels to open, which does not occur even under extreme cellular hypoxia (Weiss and Hiltbrand, 1985; Decking et al., 1995, 1997), suggesting that additional modulators of K_ATP channel opening are important.

In this regard, intracellular nucleotide diphosphates are of particular importance since they favor opening of K_ATP channels even within a cytosolic environment of high ATP concentration (Ashcroft and Ashcroft, 1990; Nichols and Lederer, 1991; Weiss and Venkatesh, 1993; Findlay, 1994; Terzic et al., 1994a; Elvir-Mairena et al., 1996). However, the mechanism of this action of nucleotide diphosphates remains controversial. A conventional assumption has been that nucleotide diphosphates competitively antagonize ATP at an inhibitory binding site on the channel protein (Dunne and Petersen, 1986; Kakei et al., 1986; Misler et al., 1986; Findlay, 1987; Bokvist et al., 1991; Nichols and Lederer, 1991; Ueda et al., 1997). However, this mechanism cannot fully explain K_ATP channel opening since altered concentrations of cytosolic ATP and/or nucleotide diphosphates are not readily detectable, nor do they correlate with changes in K_ATP channel function. Moreover, nucleotide diphosphates, such as ADP or UDP, induce channel opening in the absence of ATP (Findlay, 1988; Lederer and Nichols, 1989; Tung and Kurachi, 1991; Allard and Lazdunski, 1992; Forestier and Vivaudou, 1993; Terzic et al., 1994a) and can lose their ability to antagonize ATP-dependent channel inhibition under certain operative conditions of the channel (Deutsch and Weiss, 1993; Terzic et al., 1994a). Such nonuniform regulation of K_ATP channel opening by nucleotide diphosphates has also been observed with other inhibitory ligands including sulfonylurea drugs (Venkatesh et al., 1991; Brady et al., 1996b, 1998) and diadenosine polyphosphates (Jovanovic et al., 1996, 1997). These findings suggest that an operative condi-
tion-dependent response of $K_{ATP}$ channels is a fundamental property of the channel, which may be the basis for channel opening in the presence of inhibitory ligands.

To determine whether nucleotide diphosphates induce an alteration in channel behavior that could account for the observed response of $K_{ATP}$ channels towards inhibitory ligands, we investigated the action of UDP on transitional states of the cardiac $K_{ATP}$ channel. Based on a kinetic model of channel behavior, we demonstrate that UDP drives the channel into a state that is insensitive towards inhibitory ligands. Interconversion between ligand-sensitive and -insensitive states could be interpreted using an allosteric model that predicted the outcome of the interaction between an inhibitory ligand and the $K_{ATP}$ channel in the presence of a nucleotide diphosphate. Transition of the channel into a ligand-insensitive channel state provides a means for $K_{ATP}$ channel opening even in the presence of high concentrations of inhibitory ligands within a cardiomyocyte.

**MATERIALS AND METHODS**

**Isolated Cardiomyocytes**

Ventricular myocytes were isolated by enzymatic dissociation (Alekseev et al., 1996a). Solutions were prepared based on a “low Ca²⁺ medium” containing (mM): 100 NaCl, 10 KCl, 1.2 KH₂PO₄, 5 MgSO₄, 20 glucose, 50 taurine, 10 HEPES, pH 7.2–7.3. Guinea pigs were anesthetized with pentobarbital (1 ml/100 mg body weight i.p.). After cardiotomy, the heart was retrogradely perfused (at 37°C) with: medium 199 (Sigma Chemical Co., St. Louis, MO) for 2–3 min, followed by CaCl₂ buffered low Ca²⁺ medium (pCa 7) for 80 s, and finally low Ca²⁺ medium containing pronase E (8 mg/100 ml; Serva Biochemicals, Heidelberg, Germany), protease K (1.7 mg/100 ml; Boehringer Mannheim Biochemicals, Indianapolis, IN), bovine serum albumin (0.1 g/100 ml, fraction V; Sigma Chemical Co.), and 200 μM CaCl₂. Ventricles were separated from atria and cut into small fragments (6–10 mm³) in the low Ca²⁺ medium enriched with 200 μM CaCl₂. Single cells were then isolated by stirring the tissue (at 37°C) in a solution containing pronase E and protease K supplemented with collagenase (5 mg/10 ml; Worthington Biochemical Corp., Freehold, NJ). After 10 min, the first aliquot was removed, filtered through a nylon sieve, centrifuged (at 300–400 rpm, 1 min), and washed twice. Remaining tissue fragments were reexposed to collagenase, and isolation continued for two to three such cycles. Isolated cardiomyocytes were stored in low Ca²⁺ medium with 200 μM CaCl₂. Rod-shaped cardiomyocytes with clear striations and a smooth surface were used for electrophysiological recordings. Experiments were performed with the approval of the Institutional Animal Care and Use Committee (Mayo Clinic).

**Single-Channel Recording**

Fire-polished pipettes, coated with Sylgard (resistance ~5 MΩ), were filled with “pipette solution” containing (mM): 140 KCl, 1 CaCl₂, 1 MgCl₂, 5 HEPES-KOH, pH 7.3. Cardiac cells were superfused with “internal solution” containing (mM): 140 KCl, 1 MgCl₂, 5 EGTA, 5 HEPES-KOH, pH 7.3, in the absence or presence of nucleotides (UDP or ATP) and/or glyburide (Sigma Chemical Co.), and recordings made at room temperature (20–22°C) as described (Terzic et al., 1994c; Terzic and Kurachi, 1996). Glyburide was dissolved in dimethylsulfoxide as concentrated stock solution, and the final concentration of dimethylsulfoxide was <0.1%, which did not affect $K_{ATP}$ channels. UDP (Boehringer Mannheim Biochemicals) and ATP (potassium salt; Sigma Chemical Co.) were dissolved in internal solution before use. Single-channel recordings in the inside-out configuration were monitored online on a high-gain digital storage oscilloscope (VC-6025; Hitachi Ltd., Tokyo, Japan) and stored on tape using a PCM converter system (VR-10; Instrutech Corp., Great Neck, NY). Data were reproduced, low-pass filtered at 4 kHz (~3 dB) by a Bessel filter (902; Frequency Devices Inc., Haverhill, MA), sampled at 80-μs rate, and further analyzed using “BioQuest” software (Alekseev et al., 1997a, 1997b).

**Analysis of Channel Activity**

The threshold for judging the open state of $K_{ATP}$ channels was set at half single channel amplitude. The degree of channel activity was assessed by digitizing segments of current records, expressed as $n_P$, where $n$ represents the number of channels in the patch and $P$, the probability of each channel to be open.

Kinetic schemes for cardiac $K_{ATP}$ channels are commonly represented by one open and two closed states (Kakei and Noma, 1984). Herein, in prolonged records of single channel activity (under symmetrical $K^+$ concentration and negative membrane potential), distribution of total dwell time could be best fit by three exponentials, thus requiring consideration of an additional closed state as proposed for more detailed schemes of $K_{ATP}$ channel behavior (Gillis et al., 1989; Furukawa et al., 1993). Accordingly, channel kinetic analysis was performed based upon a four-state kinetic scheme with three forward and three backward rate constants:

$$C_1 \xrightarrow{k_{10}} O \xrightarrow{k_{01}} C_2 \xrightarrow{k_{20}} C_3,$$

where transitions between the open (O) and the first closed ($C_1$) state represent transitions within a burst (intraburst kinetics), whereas transitions between the open (O) and the second ($C_2$) and third ($C_3$) closed states define interburst kinetics.

Rate constants (see Scheme 1) were calculated based on parameters obtained from the fit of separated distributions of intraburst and interburst closed times, since relative areas under exponentials that correspond to interburst transitions were negligible compared with intraburst events. A critical time ($t_{cutoff}$) was used to define the maximal duration of an event that could still be interpreted as closure within a burst of channel activity and was used to construct distributions of open and closed events within and between bursts. The value for $t_{cutoff}$ ≥ 3.5 ms was determined based upon the relationship (Gillis et al., 1989):

$$a_1 \exp (-t_{cutoff}/\tau_1) = a_2 \left[ \exp (-t_{cutoff}/\tau_2) - \exp (-t_{cutoff}/\tau_3) \right],$$

where $\tau_1$ is the time constant of closed intervals within a burst; $\tau_2$ is the time constant of closed intervals between bursts; $a_1$ and $a_2$ are areas of exponential fits corresponding to $\tau_1$ and $\tau_2$, respectively; and $t_{cutoff}$ is the “dead time;” i.e., time of underestimated events that equals the double sampling rate (~100 μs). Fitting closed and open time distributions by the sum of exponentials was carried out using minimization of the $\chi^2$ criterion with the Nelder-Mead method of deformed polyhedron (Alekseev et al., 1996b).
Characteristic open time ($\tau_o$) was interpreted as:

$$\tau_o = 1 / (k_{o1} + k_{o2}).$$

Characteristic intraburst closed time ($\tau_{c,1}$), corresponding to gaps within burst, was interpreted as the lifetime in the C1 state:

$$\tau_{c,1} = 1 / k_{10}.$$

Similarly, the first characteristic interburst closed time ($\tau_{c,2}$) was interpreted as the lifetime in the C2 state:

$$\tau_{c,2} = 1 / (k_{20} + k_{23}).$$

Finally, the second characteristic interburst closed time ($\tau_{c,3}$) can be approximated as (see Sakmann and Trube, 1984; Gillis et al., 1989):

$$\tau_{c,3} = 1 / k_{23} \left( 1 + k_{23} / k_{20} \right).$$

In addition, the number of intraburst closures per burst was expressed as:

$$N_{IB} / N_B = k_{o1} / k_{o2}$$

where $N_{IB}$ is the number of events within a burst, and $N_B$ is the number of bursts or of gaps between burst, distribution of which was defined by $\tau_{c,3}$ and $\tau_{c,5}$, with representative relative areas under each exponent, $a_2$ and $a_3$ ($a_2 + a_3 = 1$), respectively. Therefore:

$$a_2 = k_{20} / k_{23}$$

Eqs. 2–7 were then solved for each of the three forward and three backward rates of transition (see Scheme 1):

$$k_{10} = 1 / \tau_{c,1}$$
$$k_{o1} = N_{IB} / (\tau_{c,1} \left( N_{IB} + N_B \right))$$
$$k_{o2} = 1 / (\tau_{c,2} \left[ N_{IB} / N_B + 1 \right])$$
$$k_{20} = a_2 / \tau_{c,2}$$
$$k_{23} = 1 / (\tau_{c,2} \left[ a_2 / a_3 + 1 \right])$$
$$k_{32} = 1 / (a_2 - \tau_{c,3} - \tau_{c,2}).$$

Calculated rates of transition were used as a quantitative tool to describe the effect of nucleotides and sulfonylurea drugs on K_{ATP} channel kinetics.

Results were expressed as mean ± SEM; $n$ refers to the number of myocytes used in each analysis.

RESULTS

UDP Directs Cardiac K_{ATP} Channel Activity Towards Intraburst Transitions

The single channel behavior of cardiac K_{ATP} channels is characterized by clustering of channel openings in groups (bursts) separated by prolonged closures (gaps between bursts; Fig. 1 A). Within a burst, distributions of closed and open times were fitted by corresponding single exponents (characteristic times $\tau_{c,1} = 0.48 \pm 0.03$ ms and $\tau_o = 2.21 \pm 0.18$ ms, respectively; $n = 5$; Fig. 1 B, left). Between bursts, distribution of gaps required at least two exponents (Fig. 1 C, left). Thus, in addition to the open and closed channel states that define intraburst ($C_1 \leftrightarrow O$) transitions, two closed states were required to describe interburst ($\leftrightarrow C_2 \leftrightarrow C_3$) K_{ATP} channel behavior (Fig. 1 D, left).

The nucleotide diphosphate UDP (1 mM) eliminated gaps between bursts, promoting the channel to operate within a sustained burst (Fig. 1 A; Table I). UDP did not change the distribution of open times (Table I) that could be fitted by a single exponent (characteristic time $\tau_o = 2.36 \pm 0.22$ ms; $n = 4$; Fig. 1 B, right), not statistically different from the value obtained in the absence of UDP ($P < 0.05$; see above). However, since UDP eliminated gaps between bursts, the total distribution of closed times became a single exponent (Fig. 1 C, right) with a characteristic time $\tau_{c,1} = 0.48 \pm 0.04$ ms ($n = 4$). This value was identical to the parameter defining closures within bursts of channel activity in the absence of UDP ($P < 0.05$; see above). In terms of the four-state linear scheme of K_{ATP} channel activity, UDP reduced channel operation to one closed and one open state with a rate of transition (Fig. 1 D, right), identical to the rate of intraburst transition measured in the absence of UDP (Fig. 1 D, left). Thus, UDP directed the K_{ATP} channel to operate exclusively within intraburst transitions (Fig. 1 D, right).

ATP and Glyburide Affect K_{ATP} Channel Behavior Outside Intraburst Transitions

In the absence of ligands, K_{ATP} channel activity displayed intraburst and interburst transitions (Fig. 2, a1 and b1). ATP (100 $\mu$M; Fig. 2 A, a2) or glyburide (1 $\mu$M; Fig. 2 B, b2) blocked K_{ATP} channel activity without affecting single channel amplitude ($n = 48$). Neither ATP ($n = 5$) nor glyburide ($n = 4$) significantly changed mean closed ($\tau_{c,1}$) and mean open ($\tau_o$) times that define intraburst kinetics (Table I). However, both inhibitory ligands did prolong the fast ($\tau_{c,2}$) and slow ($\tau_{c,3}$) characteristic times that define the distribution of gaps between bursts (Table I) and increased the number of prolonged channel closures (Table I; relative areas $a_1$ and $a_2$). This was associated with a significant change in transition rates defining interburst, without a change in rates ($k_{10}$ and $k_{o1}$) defining intraburst ($C_1 \leftrightarrow O$; Fig. 2) transitions. Specifically, ATP (100 $\mu$M) promoted escape of the channel from intraburst transitions to the C2 closed state by increasing the $k_{20}$ rate from 0.55 to 28 s^{-1}, and delayed initiation of a burst by decreasing the backward $k_{23}$ rate from 70 to 22 s^{-1} (Fig. 2 A). Similarly, but to a lesser extent than ATP, glyburide (1 $\mu$M) increased by threefold the $k_{20}$ rate, and decreased by over twofold the backward $k_{23}$ rate (Fig. 2 B). Consequently, the mean duration of a burst (2,210 ms, $n_{burst} = 23$ and 2,380 ms, $n_{burst} = 14$ in the absence of ATP and glyburide, respectively) was reduced by
each of the inhibitory ligands (56 ms, $n_{\text{burst}} = 255$ and 850 ms, $n_{\text{burst}} = 31$ in the presence of ATP and glyburide, respectively).

In agreement with experimental data, calculated mean burst duration, using rates defining intraburst transitions and the rate leading away from these transitions (Sakmann and Trube, 1984):

$$\sigma_{\text{burst}} = \frac{k_{10} + k_{20}}{k_{12}k_{21}}$$  \hspace{1cm} (8)

was 2,200 vs. 44 ms in the absence and presence of ATP, and 2,500 vs. 960 ms in the absence and presence of glyburide.

Since the lifetime the channel spends in a specific state is defined by the reciprocal of the sum of transition rates that lead away from this state:

$$\sigma_{C_2} = \frac{1}{k_{20} + k_{23}}; \quad \sigma_{C_3} = \frac{1}{k_{32}}$$  \hspace{1cm} (9)

the lifetime the $K_{\text{ATP}}$ channel spent in $C_2$ or $C_3$, in the presence of ATP (100 $\mu$M), was 12.2 and 175 ms, respectively. Although these values were within the range of values obtained in the absence of ATP ($\sigma_{C_2} = 9.1 \pm 1.4$ ms, and $\sigma_{C_3} = 207 \pm 84$ ms; $n = 4$), ATP by accelerating $k_{23}$ and by reducing $k_{20}$ rates (Fig. 2 A) promoted the channel to operate within $C_2 \leftrightarrow C_3$ closed states, away from intraburst transitions. Thereby, ATP significantly increased the combined lifetime the $K_{\text{ATP}}$ channel spent within interburst transitions (Sakmann and Trube, 1984):

$$\sigma_{C_{2,3}} = \frac{k_{13} + k_{21}}{k_{12}k_{20}}$$  \hspace{1cm} (10)

from 87.8 ms in the absence to 524 ms in the presence of ATP (Fig. 2 A).

Glyburide (1 $\mu$M) reduced the lifetime in either the $C_2$ (from 9.7 to 20 ms) or the $C_3$ (from 417 to 833 ms) closed states (Fig. 2 B). The mean lifetime ($\sigma_{C_{2,3}}$) the channel spent within $C_2 \leftrightarrow C_3$ closed states increased from 132 ms in the absence to 589 ms in the presence of glyburide (Fig. 2 B). Thus, ATP and glyburide, despite apparent differences in the mechanism of chan-

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**Figure 1.** UDP induces loss of interburst transition. (A) Single $K_{\text{ATP}}$ channel records (80 s in duration) in the absence (top) and presence (bottom) of 1 mM UDP. Records under both conditions are presented on compressed and extended time scales. Zero-current level is indicated by 0 pA in the compressed, and by a dotted line in the extended time record. (B, left) In the absence of UDP, open and closed (inset) time distributions of intraburst events were fitted by single exponents (solid lines) with characteristic times $\tau_o$ and $\tau_{c,1}$. (right) Distribution of openings within a burst in the presence of 1 mM UDP. (C, left) In the absence of UDP, distribution of gaps between bursts was fitted by the sum of two exponents (solid line) with characteristic times $\tau_{c,2}$ and $\tau_{c,3}$. Dashed lines correspond to individual exponents. (right) In the presence of UDP, there were no interburst events, and the distribution of closed times could be fitted by a single exponent with a characteristic time, $\tau_{c}$, essentially identical to the mean closed time for intraburst events ($\tau_{c,1}$) obtained in the absence of UDP. Kinetic scheme constructed based on calculated rates of transitions (in s$^{-1}$) using Eq. 8 in the absence (left) and presence (right) of 1 mM UDP. Holding potential was $-60$ mV.
nel inhibition, act outside the intraburst $C_1 \leftrightarrow O$ transition, shorten burst duration, and prolong the time the K$_{ATP}$ channel remains within $C_2$ and $C_3$ closed states.

**UDP Prevents Ligand Inhibition of K$_{ATP}$ Channels by Favoring Intraburst Activity**

In the absence of UDP, K$_{ATP}$ channel activity exhibited intraburst and interburst transitions (Fig. 3, A and B, Table I) and was sensitive to the inhibitory action of ATP (200 µM). Washout of ATP restored channel activity, which was directed towards intraburst transition by 1 mM UDP (Fig. 3, A and C). Intraburst forward and backward rates of transition were similar before (2,000 and 434 s$^{-1}$), and after (2,000 and 420 s$^{-1}$) addition of UDP and ATP. However, UDP eliminated gaps between bursts in all patches so tested ($n = 4$), which induced an apparent insensitivity of the K$_{ATP}$ channel towards ATP (Fig. 3 A).

The effect of UDP was also tested in the presence of glyburide, a nonnucleotide inhibitory ligand of the channel. As in the case of ATP (Fig. 3 A), maintenance of channel activity within intraburst transitions by UDP also prevented glyburide (5 µM) to block K$_{ATP}$ channel activity (Fig. 3 D; $n = 6$). Thus, regardless of the structure of the inhibitory ligand, UDP could apparently shield the K$_{ATP}$ channel from ATP or glyburide by “trapping” the channel within ligand-insensitive transitions.

**UDP Is Not the Sole Determinant of Sensitivity to Inhibitory Ligands**

The efficacy with which UDP antagonizes ATP- and glyburide-inhibitory gating has been reported to vary with the operative condition of the K$_{ATP}$ channel (Terzic et al., 1994a; Brady et al., 1998). As shown in Fig. 4 A, sustained spontaneous K$_{ATP}$ channel activity was only partially sensitive to ATP (300 µM) in the presence of UDP (2 mM), yet fully sensitive in the absence of UDP ($n = 10$). With channel “rundown,” UDP restored channel activity but could no longer antagonize ATP (Fig. 4 A, see also Terzic et al., 1994a). Similarly, after rundown, UDP induced channel opening that was inhibited by glyburide (1 µM; Fig. 4 B; $n = 5$). However, restoration of spontaneous channel activity, by pretreatment with MgATP (5 mM), was associated with return of UDP-induced antagonism of glyburide inhibition (Fig. 4 B, see also Brady et al., 1998). Thus, channel rundown ap-

**Table I**

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<tr>
<th>Parameters of Open and Closed Time Distributions of K$_{ATP}$ Channel Activity Obtained under Different Experimental Conditions</th>
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<tr>
<td>Number of events within burst ($N_{IB}$)</td>
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<td>Number of events between burst ($N_B$)</td>
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<td>Gaps within a burst ($t_{c,1}$), ms</td>
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<td>Gaps between bursts, fast ($t_{c,2}$), ms</td>
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<td>Gaps between burst, slow ($t_{c,3}$), ms</td>
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<td>Relative area of $t_{c,2}$ ($a_2$)</td>
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<td>Relative area of $t_{c,3}$ ($a_3$)</td>
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<td>Open time ($t_o$), ms</td>
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**Figure 2.** Inhibitory ligands act on K$_{ATP}$ channels outside intraburst transition. Portions of original single channel records in the absence ($a_1$ and $b_1$) and presence ($a_2$) of 100 µM ATP or 1 µM glyburide ($b_2$). Corresponding kinetic schemes with calculated rates of transitions (in s$^{-1}$, Eq. 8) are provided for each record in the absence and presence of ATP (A) and in the absence and presence of glyburide (B). Holding potential was −60 mV throughout.
pears to decrease the efficacy with which UDP antagonizes the action of inhibitory ligands.

**Rundown Prevents UDP from Holding K<sub>ATP</sub> Channels within Intraburst Transitions**

Progression of rundown of K<sub>ATP</sub> channel activity was associated with an increased number of prolonged closures (Fig. 5 A, top). Intraburst channel properties (τ<sub>c,1</sub> and τ<sub>c,2</sub>, Fig. 5 B, Table I) were unchanged with rundown, but fast and slow time components defining gaps between burst (τ<sub>c,2</sub> and τ<sub>c,3</sub>) were prolonged (Fig. 5 C, Table I). As the process of rundown accelerated, the k<sub>b2</sub> rate responsible for exit of the channel from a burst (from 0.52 ± 0.07 s<sup>−1</sup>, n = 4, under sustained activity to 3.1 s<sup>−1</sup> during rundown; Fig. 5), mean burst duration of partial rundown channels was shorter when compared with sustained channel activity (τ<sub>burst</sub> = 440 ms; n<sub>burst</sub> = 224 vs. τ<sub>burst</sub> = 2,456 ± 315 ms; n = 4). Furthermore, K<sub>ATP</sub> channels under partial rundown spent a significantly longer time in the C<sub>3</sub> state (Eq. 10) when compared with channels under sustained channel activity (670 ms vs. 207 ± 84 ms; n = 4), although the apparent lifetime in the C<sub>3</sub> state (12 ms vs. 9.1 ± 1.4 ms, n = 4; Eq. 10) was similar under both conditions. Thus, channel rundown directed K<sub>ATP</sub> channels away from intraburst and towards interburst transitions.

To determine the efficacy with which UDP acts on K<sub>ATP</sub> channels driven towards the C<sub>3</sub> state as rundown progresses, UDP (1 mM) was applied to partially run-
K<sub>ATP</sub> channel activity by Mg-ATP switches on the UDP-induced antagonism of glyburide-dependent channel block. A 10-min long pretreatment of rundown K<sub>ATP</sub> channels with 5 mM Mg-ATP restored spontaneous channel activity and with it the UDP-induced antagonism of glyburide-dependent channel block lost in rundown channels. In the absence of UDP, channel activity was readily inhibited by glyburide. The dotted line with original trace corresponds to the zero-current level. The dotted line corresponds to the zero-current level. (Fig. 5). UDP increased channel activity and reduced the number of channel closures (Fig. 5A, bottom). Similar to its effect on sustained K<sub>ATP</sub> channel activity, UDP did not affect intraburst kinetics; i.e., UDP did not significantly change rates defining the C<sub>1</sub> ↔ O transition (k<sub>b1</sub> and k<sub>c1</sub>) of partially rundown channels (Fig. 5A and B). However, in contrast to sustained K<sub>ATP</sub> channel activity (Fig. 1), UDP did not eliminate interburst events in rundown channels, the distribution of which remained biexponential (Fig. 5C). Also, in contrast to its effect on sustained channel activity where UDP eliminated the O ↔ C<sub>2</sub> transition (from 0.35 to ~0 s<sup>−1</sup>; Fig. 1, Table I), UDP only decreased the transition rate (k<sub>c0</sub>) associated with burst closure of partially rundown channels (i.e., the O ↔ C<sub>2</sub> transition changed from 3.1 to 2.0 s<sup>−1</sup>; Fig. 5D). Under this condition, the K<sub>ATP</sub> channel could still transit between intraburst and interburst states despite the presence of UDP (Fig. 5A and C). Mean burst duration (Eq. 9) of partially rundown channels increased in the presence of UDP from τ<sub>burst</sub> = 440 ms (n<sub>burst</sub> = 224) to τ<sub>burst</sub> = 684 ms (n<sub>burst</sub> = 125), similar to calculated values of τ<sub>burst</sub> = 412 and 631 ms in the absence and presence of UDP, respectively; Eq. 9). Acting on partially rundown channels, UDP apparently reduced the lifetime the channel spent within interburst transitions (3 and 15 ms for C<sub>2</sub> and C<sub>3</sub>, respectively; Fig. 5A; Eq. 10). Thus, under such conditions, although UDP still directed partially rundown channels towards intraburst ligand-insensitive transitions, the nucleotide diphosphate could not maintain the channel within a ligand-insensitive state.

**Allosteric Model of Ligand/Channel Interaction**

It has been reported that UDP shifts to the right the concentration–response curve to ATP (Terzic et al., 1994a), whereas it completely eliminates the sensitivity of the K<sub>ATP</sub> channel to glyburide (Brady et al., 1998). Based on the kinetic model, it was not possible to quantitatively predict the different response of K<sub>ATP</sub> channels to inhibitory ligands. Therefore, an allosteric model of ligand/protein interaction (Monod et al., 1965), previously applied to analyze ligand regulation of ion channels (Karlin, 1967; Hosoya et al., 1997; Tibbs et al., 1997), was used. The features of such allosteric models are that (a) the allosteric protein (i.e., the channel complex) interconverts within two distinct conformational states: ligand-sensitive (S) and -insensitive (I); (b) the channel complex possesses two sets of binding sites (n and m), one for the inhibitor (A), and the second for the activator (B); (c) each set of binding sites is equivalent within a state, but exhibits different microscopic dissociation constants (K<sub>A</sub>S or K<sub>B</sub>S in S and K<sub>A</sub>I or K<sub>B</sub>I in I) between states; and (d) binding of an inhibitor or an activator shifts, in opposite directions, the equilibrium between the two conformational states and thus increases the fraction of total protein (chan-
channel complex) in the state with higher affinity for a particular ligand and decreases the apparent (macroscopic) affinity towards the other ligand.

The equilibrium constant \( L \) in the absence of a ligand was expressed by the ratio between the channel lifetime in ligand-insensitive (i.e., mean burst duration; Eq. 9) and -sensitive (i.e., lifetime spent in \( C_2 \) plus \( C_3 \); Eq. 11) states:

\[
L = \frac{\sigma_{\text{burst}}}{\sigma_{C2,3}} = \frac{(k_{30} + k_{03}) k_{21} k_{01}}{(k_{31} + k_{13}) k_{01} k_{02}}.
\]  

From our experimental data (i.e., Figs. 1 and 2), calculation of \( L \) revealed a value in the range of 150–200 under spontaneous channel activity. This indicates that under sustained spontaneous channel activity the equilibrium between ligand-sensitive and -insensitive states is significantly shifted towards ligand-insensitive states, which corresponds to a prolonged burst of channel activity.

Channel inhibition is a function of the fraction of the protein in the \( S \) state:

\[
S = \frac{1}{1 + L \left[ \frac{1 + d \beta}{1 + \beta} \right]^{\alpha} \left[ \frac{1 + d \epsilon}{1 + \epsilon} \right]^{n}}
\]  

where \( d = K_{B,S} / K_{B,P} \), \( \beta = [B]/K_{B,S} \), \( \epsilon = K_{A,S}/K_{A,P} \), and \( \alpha = [A]/K_{A,S} \). For \( A \), the allosteric inhibitor (e.g., glyburide or ATP), \( \epsilon < 1 \). For \( B \), the allosteric activator (i.e., UDP), \( d > 1 \).

In the absence of UDP, the concentration dependence of \( K_{\text{ATP}} \) channel inhibition by glyburide (\( A \); Fig. 6...
Alekseev et al. was well fitted by the \( I-S \) function (Eq. 13) using the following parameters: \( L = 200; K_{A,S} = 1 \text{ nM}, K_{A,I} = 1 \text{ mM}, n = 1 \) (Fig. 6 A, solid line). In the presence of 1 mM UDP, the observed loss of sensitivity of the \( K_{\text{ATP}} \) channel towards the sulfonylurea (Fig. 6 A, ○) was well described by the allosteric model (\( L = 200; K_{A,S} = 1 \text{ nM}, K_{A,I} = 1 \text{ mM}, n = 1; K_{B,S} = 3.5 \text{ mM}, K_{B,I} = 0.1 \text{ mM}, m = 4 \)). The model predicts that at millimolar concentrations of the activator (UDP) the effect of glyburide on \( K_{\text{ATP}} \) channel activity will be fully antagonized even at tens of micromoles of the inhibitor (Fig. 6 A, solid line).

A. ○) was well fitted by the \( I-S \) function (Eq. 13) using the following parameters: \( L = 200; K_{A,S} = 1 \text{ nM}, K_{A,I} = 1 \text{ mM}, n = 1 \) (Fig. 6 A, solid line). In the presence of 1 mM UDP, the observed loss of sensitivity of the \( K_{\text{ATP}} \) channel towards the sulfonylurea (Fig. 6 A, ○) was well described by the allosteric model (\( L = 200; K_{A,S} = 1 \text{ nM}, K_{A,I} = 1 \text{ mM}, n = 1; K_{B,S} = 3.5 \text{ mM}, K_{B,I} = 0.1 \text{ mM}, m = 4 \)). The model predicts that at millimolar concentrations of the activator (UDP) the effect of glyburide on \( K_{\text{ATP}} \) channel activity will be fully antagonized even at tens of micromoles of the inhibitor (Fig. 6 A, solid line).

In the absence of UDP, the concentration dependence of \( K_{\text{ATP}} \) channel inhibition by ATP (A; Fig. 6 B, □) was also well fitted by the \( I-S \) function (Eq. 13) using the following parameters: \( L = 200; K_{A,S} = 8.5 \mu \text{M}, K_{A,I} = 70 \text{ mM}, n = 3.6 \) (Fig. 6 B, curve 1). However, in the presence of UDP (5 mM; \( K_{B,S} = 3.5 \text{ mM}, K_{B,I} = 0.1 \text{ mM}, m = 4 \)), the allosteric model predicted a rightward shift but failed to precisely fit (Fig. 6 B, curve 2) the experimentally obtained data defining the concentration response of ATP-induced \( K_{\text{ATP}} \) channel inhibition under this condition (Fig. 6 B, ▼). Since varying the number of binding sites, cooperativity, and/or dissociation constants for ATP did not improve the fit, we further developed the model taking into account the existence of an additional presumed binding site for ATP not affected by nucleotide diphosphate regulation (Tucker et al., 1997; Ueda et al., 1997). Therefore, we added to the \( I-S \) function (Eq. 13) an additional allosteric regulation-independent inhibitory process to account for both mechanisms of ATP inhibitory action:
responsive behavior of KATP channels to inhibitory cande, 1989; Deutsch and Weiss, 1993). Thus, the use could increase the affinity of ligands for the ligand interaction. For instance, rundown could also alter other parameters of channel/ligand interaction. In contrast to spontaneous channel activity, where UDP increased $L \rightarrow \infty$ (by $k_{\text{off}} \rightarrow 0$), under partial rundown UDP elevated $L$ only up to 70–90 (i.e., Fig. 5). However, such a change in $L$ was not sufficient to restore the experimentally obtained channel sensitivity towards ATP and glyburide (Terzic et al., 1994; Brady et al., 1998). This could indicate that besides the effect on $L$, rundown could also alter other parameters of channel/ligand interaction. For instance, rundown could decrease the affinity of ligands for the $I$ state, which would promote the effect of inhibitors (Thuringer and Escande, 1989; Deutsch and Weiss, 1993). Thus, the use of the allosteric model could explain the nonuniform responsive behavior of $K_{\text{ATP}}$ channels to inhibitory ligands in the presence of UDP depending on the operative condition of the channel.

Discussion

The present study demonstrates that cardiac $K_{\text{ATP}}$ channels can be directed to operate within ligand-insensitive conformational states. The switch into ligand-insensitive behavior was induced by the nucleotide diphosphate, UDP. Interconversion between ligand-sensitive and -insensitive states represents a novel mechanism of $K_{\text{ATP}}$ channel regulation. The property of a nucleotide diphosphate to direct cardiac $K_{\text{ATP}}$ channels towards a state that is insensitive towards inhibitory ligands could provide a mechanistic basis for channel opening in the presence of inhibitory concentrations of ATP within an intact cell.

Ligand–Channel Interaction and Kinetic Model

To distinguish between conformational transitions that define $K_{\text{ATP}}$ channel activity, we applied a linear kinetic model used previously (Sakmann and Trube, 1984; Gillis et al., 1989; Nichols et al., 1991; Furukawa et al., 1993; Takano and Noma, 1993). This entropic model does not describe all conformations through which a channel transits, but it does allow description of end points of sequential conformational transitions accessible to direct measurement. Although this simplified model was developed for inward $K_{\text{ATP}}$ channel current as recorded under present experimental conditions (with symmetrical K$^+$ solutions and at a holding potential of $-60 \text{ mV}$), intraburst and interburst transitions can also be distinguished for outward $K_{\text{ATP}}$ channel currents despite more complex intraburst kinetics (Zilberter et al., 1988; Larsson et al., 1993; Alekseev et al., 1997b). Herein, we found that the inhibitory ligands, ATP and glyburide, inhibited $K_{\text{ATP}}$ channel activity by acting upon conformational states that define interburst behavior without affecting intraburst channel transitions. This is in agreement with previous studies that have also shown that inhibitory ligands target specific rates of channel kinetics (Gillis et al., 1989; Qin et al., 1989; Nichols et al., 1991; Takano and Noma, 1993; Benz and Kohlhardt, 1994; Smith et al., 1994). We further found that UDP could keep the channel within a burst, preventing interburst transitions. Therefore, the observed effect of UDP to antagonize channel inhibition by ATP and glyburide could be attributed to the limitation of channel behavior within ligand-insensitive intraburst conformational transitions. Such a mechanism could explain the altered responsiveness of cardiac $K_{\text{ATP}}$ channels towards ATP and sulfonylureas observed in the presence of nucleotide diphosphates (Nichols and Lederer, 1991; Venkatesh et al., 1991; Virag et al., 1993; Findlay, 1994; Terzic et al., 1994a; Brady et al., 1998). Based on the kinetic model used, the present study provides evidence that the response of the cardiac $K_{\text{ATP}}$ channel depends not only on the concentration of an inhibitor, but also on the lifetime the channel spends within ligand-sensitive states. This concept may not be limited to UDP. Indeed, it has been shown that other agents, such as potassium channel openers (Fan et al., 1990; Terzic et al., 1994b), intracellular protons (Vivaudou and Forestier, 1995; Alekseev et al., 1997a), cytoskeleton disrupters (Brady et al., 1996a), or channel trypsinization (Deutsch and Weiss, 1994), which also promote the $K_{\text{ATP}}$ channel to operate within a burst, decrease the sensitivity of the channel towards inhibitory ligands. Furthermore, combined application of ADP and the opener diazoxide potentiated the ability of these agents to antagonize ATP inhibition of $K_{\text{ATP}}$ channels by prolonging the lifetime the channel spends within a burst (Larsson et al., 1993). Conversely, ATP and related nucleotides that direct the $K_{\text{ATP}}$ channel to operate within interburst transitions were shown to enhance the sensitivity of $K_{\text{ATP}}$ channels towards sul-
Application of an Allosteric Model to the Regulation of \( K_{\text{ATP}} \) Channels

The dual nature of \( K_{\text{ATP}} \) channel behavior, in terms of ligand-sensitive and -insensitive states, drew parallelism with interconversion of an allosteric protein between two significant conformational states with different affinities to ligands (Monod et al., 1965; Karlin, 1967). This allosteric model predicted the observed change in the ATP- and glyburide-dependent inhibitory gating of the channel induced by a UDP-mediated shift in the equilibrium towards the ligand-insensitive state of the \( K_{\text{ATP}} \) channel. The difference in the microscopic affinities for the two conformational states (10^6 for glyburide and \( \sim 10^4 \) for ATP) predicted by the present allosteric model is consistent with the existence of ligand-insensitive and -sensitive states of the channel. In fact, the allosteric model predicted that at millimolar concentrations of UDP the cardiac \( K_{\text{ATP}} \) channel loses its sensitivity towards glyburide. This is in accord with previous studies that have established that under spontaneous \( K_{\text{ATP}} \) channel activity, nucleotide diphosphates, such as UDP or ADP, antagonize sulfonylurea-induced channel inhibition (Venkatesh et al., 1991; Virag et al., 1993; Brady et al., 1998). Although it is difficult to compare microscopic with apparent dissociation constants, high and low affinities for sulfonylurea binding have been previously reported (Fosset et al., 1988; Aguilar-Bryan et al., 1992). Thus, due to the negligible affinity of glyburide towards the ligand-insensitive (I) state, UDP by shifting the equilibrium of the \( K_{\text{ATP}} \) channel towards this particular state could effectively antagonize the effect of the sulfonylurea. In the case of ATP, for which the model predicts an additional, nucleotide diphosphate-independent, ATP-inhibitory channel gating, UDP could produce only a rightward shift in the concentration response curve of ATP-induced channel inhibition, as previously experimentally observed with UDP (Terzic et al., 1994a) or other nucleotide diphosphates such as ADP (Findlay, 1988; Lederer and Nichols, 1989). In contrast to UDP, channel rundown shifted the equilibrium towards the ligand-sensitive state of the \( K_{\text{ATP}} \) channel. This increased sensitivity of the channel towards inhibitory ligands is in accord with experimental findings that have shown that rundown enhances the inhibitory action of ATP on cardiac \( K_{\text{ATP}} \) channel activity (Thueringer and Escande, 1989; Deutsch and Weiss, 1993). Since rundown is believed to be associated with changes in the phosphorylation status of the \( K_{\text{ATP}} \) channel or associated proteins (Trube and Hescheler, 1984; Findlay and Dunne, 1986; Findlay, 1987; Ohno-Shosaku et al., 1987; Takano et al., 1990; Furukawa et al., 1996; Hilgeman and Ball, 1996), the equilibrium between the S and I channel states may be dependent upon a phosphorylation process. Treatment of rundown membrane patches with Mg-ATP (but not with ATP alone or with nonhydrolyzable ATP analogs), through presumed “re-phosphorylation” of channel proteins, restored spontaneous cardiac \( K_{\text{ATP}} \) channel activity, and with it the efficacy of UDP to antagonize ATP- and glyburide-induced channel inhibition (see also Terzic et al., 1994a; Brady et al., 1998).

Relevance to the Molecular Structure of \( K_{\text{ATP}} \) Channels

Results predicted by the allosteric model applied herein to the native cardiac \( K_{\text{ATP}} \) channel are in agreement with the reported structure and stoichiometry of the recombinant \( K_{\text{ATP}} \) channel complex (Inagaki et al., 1995, 1996; Isomoto et al., 1996; Clement et al., 1997; Tucker et al., 1997). This channel is a heteromultimer that combines four Kir6.2 and four SUR subunits into an octamer (Clement et al., 1997; Inagaki et al., 1997). It has been suggested that ATP binds to both the pore-forming Kir6.2 (Tucker et al., 1997) and the regulatory SUR (Bernardi et al., 1992; Ueda et al., 1997) subunits. In view of this, the requirement of two sets of binding sites for ATP could be interpreted to indicate two separate ATP-binding sites on each subunit of the channel complex. The allosteric model further predicts four binding sites for UDP on the \( K_{\text{ATP}} \) channel complex. This apparently correlates with the previously observed binding of a nucleotide diphosphate to only one of the channel subunits, the SUR subunit (Bernardi et al., 1992; Nichols et al., 1996; Gribble et al., 1997; Trapp et al., 1997; Tucker et al., 1997; Ueda et al., 1997). The binding of sulfonylureas to the \( K_{\text{ATP}} \) channel is also presumed to occur on the SUR subunit (Aguilar-Bryan et al., 1995; Inagaki et al., 1995, 1996; Clement et al., 1997). However, in terms of the allosteric model and in contrast to nucleotides, \( K_{\text{ATP}} \) channel regulation by glyburide was characterized by lack of cooperativity (see also Venkatesh et al., 1991).

Intraburst kinetics that define ligand-insensitive transitions are apparently associated with conformational fluctuation of Kir6.2 itself (Alekseev et al., 1997b; Tucker et al., 1997), whereas interburst kinetics are modulated by association of Kir6.2 with the SUR subunit (Inagaki et al., 1996). In view of the proposed structure of the \( K_{\text{ATP}} \) channel complex (Inagaki et al., 1995, 1996, 1997; Clement et al., 1997; Tucker et al., 1997), the kinetic and allosteric properties of channel behavior may provide the basis for a mechanistic model of the UDP-induced changes in the ATP- and glyburide-dependent regulation of \( K_{\text{ATP}} \) channel gating (Fig. 7). Such a model implies the existence of two inhibitory gating pathways. The first, mediated through binding of inhibi-
Allosteric Regulation of K<sub>ATP</sub> Channels

Tertiary ligands to the SUR subunit, appears to be sensitive to UDP regulation. The second, mediated through binding of ATP to Kir6.2, appears to be insensitive to UDP regulation. Disruption of the first inhibitory gating pathway by UDP switches the channel into sulfonylurea-insensitive behavior and decreases the channel sensitivity towards ATP (Fig. 7). Phosphorylation(s) of the channel protein can apparently restore the ability of UDP to disrupt the first inhibitory pathway lost after rundown of channel activity.

Concluding Remarks

Although the present study used UDP as a nucleotide diphosphate, the observed effect on K<sub>ATP</sub> channel behavior may also be attributable to other nucleotide diphosphates, such as ADP. In contrast to UDP, the presence of the adenine moiety could make the interpretation of the effect of ADP more complex due to possible competitive interaction of ADP with an ATP-binding site (Ueda et al., 1997). Despite this, similar effects of ADP (MgADP) in modulating the K<sub>ATP</sub> channel inhibitory gating and postrundown channel behavior have previously been demonstrated (Dunne and Petersen, 1986; Tung and Kurachi, 1991; Venkatesh et al., 1991; Weiss and Venkatesh, 1993; Findlay, 1988, 1994; Elvir-Mairena et al., 1996), as well as the ability of MgADP to prolong burst duration (Larsson et al., 1993).

The property of cardiac K<sub>ATP</sub> channels to interconvert between ligand-sensitive and -insensitive states, described herein, resembles other ion channels that also show differential sensitivity towards ligands depending on their operative state, including “use-dependent” blockade of Na<sup>+</sup> and Ca<sup>2+</sup> channels (Lee and Tsien, 1983; Hill et al., 1989; Ragsdale at al., 1994; Nuss et al., 1995). Entry of the K<sub>ATP</sub> channel into a ligand-insensitive state by a nucleotide diphosphate could provide a basis for cardiac K<sub>ATP</sub> channel opening during hypoxia or ischemia despite rather constant levels of ATP within the cell.

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