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 leads 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 IC_{50} 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., 1994*d*; 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., 1994*a*) 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., 1994*a*). 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., 1996*b*, 1998) and diadenosine polyphosphates (Jovanovic et al., 1996, 1997). These findings suggest that an operative condi-

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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., 1996*a*). Solutions were prepared based on a "low Ca^{2+} medium" containing (mM): 100 NaCl, 10 KCl, 1.2 KH_2PO_4, 5 $MgSO_4$, 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 Ca^{2+} EGTA-buffered low Ca^{2+} medium (pCa 7) for 80 s, and finally low Ca^{2+} medium containing pronase E (8 mg/100 ml; Serva Biochemicals, Heidelberg, Germany), proteinase 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 $CaCl_2$. Ventricles were separated from atria and cut into small fragments (6–10 mm^3) in the low Ca^{2+} medium enriched with 200 μM $CaCl_2$. Single cells were then isolated by stirring the tissue (at 37°C) in a solution containing pronase E and proteinase 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 Ca^{2+} medium with 200 μM $CaCl_2$. 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 $CaCl_2$, 1 $MgCl_2$, 5 HEPES-KOH, pH 7.3. Cardiac cells were superfused with "internal solution" containing (mM): 140 KCl, 1 $MgCl_2$, 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., 1994*c*; 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., 1997*a*, 1997*b*).

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 nP_o, where n represents the number of channels in the patch and P_o 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 exponents, 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:

$$C_1 \underset{k_{01}}{\overset{k_{10}}{\rightleftharpoons}} O \underset{k_{20}}{\overset{k_{02}}{\rightleftharpoons}} C_2 \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} C_3, \qquad (1)$$

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 exponents 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 $t_{cutoff} \geq 3.5$ ms was determined based upon the relationship (Gillis et al., 1989):

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

where τ_1 is the time constant of closed intervals within a burst; τ_2 is the time constant of closed intervals between bursts; a_1 and a_2 are areas of exponential fits corresponding to τ_1 and τ_2, respectively; and t_d 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 exponents was carried out using minimization of the χ^2 criterion with the Nelder-Meed method of deformed polyhedron (Alekseev et al., 1996*b*).

Characteristic open time (τ_o) was interpreted as:

$$\tau_o = 1/(k_{01} + k_{02}). \quad (2)$$

Characteristic intraburst closed time ($\tau_{c,1}$), corresponding to gaps within burst, was interpreted as the lifetime in the C_1 state:

$$\tau_{c,1} = 1/k_{10}. \quad (3)$$

Similarly, the first characteristic interburst closed time ($\tau_{c,2}$) was interpreted as the lifetime in the C_2 state:

$$\tau_{c,2} = 1/(k_{20} + k_{23}). \quad (4)$$

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

$$\tau_{c,3} = \frac{1}{k_{32}}\left(1 + \frac{k_{23}}{k_{20}}\right) + \frac{1}{k_{20}}. \quad (5)$$

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

$$\frac{N_{1B}}{N_B} = \frac{k_{01}}{k_{02}} \quad (6)$$

where N_{IB} is the number of events within a burst, and N_B is the number of bursts or of gaps between bursts, distribution of which was defined by $\tau_{c,2}$ and $\tau_{c,3}$, with representative relative areas under each exponent, a_2 and a_3 ($a_2 + a_3 = 1$), respectively. Therefore:

$$\frac{a_2}{a_3} = \frac{k_{20}}{k_{23}}. \quad (7)$$

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

$$\begin{aligned} k_{10} &= 1/\tau_{c,1} \\ k_{01} &= N_{1B}/(\tau_0 [N_{1B} + N_B]) \\ k_{02} &= 1/(\tau_0 [N_{1B}/N_B + 1]) \\ k_{20} &= a_2/\tau_{c,2} \\ k_{23} &= 1/(\tau_{c,2} [a_2/a_3 + 1]) \\ k_{32} &= 1/(a_2 \cdot \tau_{c,3} - \tau_{c,2}). \end{aligned} \quad (8)$$

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 are 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, a_1 and b_1). ATP (100 μM; Fig. 2 *A*, a_2) or glyburide (1 μM; Fig. 2 *B*, b_2) 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 (τ_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_{01}) defining intraburst ($C_1 \leftrightarrow O$; Fig. 2) transitions. Specifically, ATP (100 μM) promoted escape of the channel from intraburst transitions to the C_2 closed state by increasing the k_{02} rate from 0.55 to 28 s^{-1}, and delayed initiation of a burst by decreasing the backward k_{20} rate from 70 to 22 s^{-1} (Fig. 2 *A*). Similarly, but to a lesser extent than ATP, glyburide (1 μM) increased by threefold the k_{02} rate, and decreased by over twofold the backward k_{20} 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

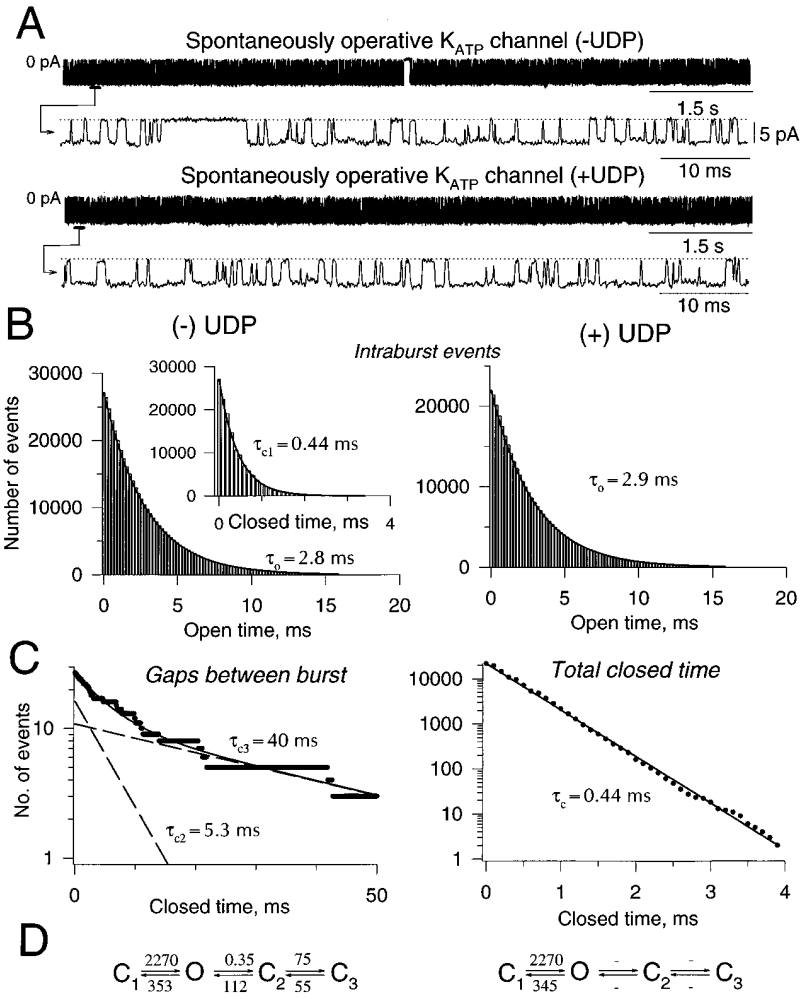

FIGURE 1. UDP induces loss of interburst transition. (*A*) Single K_{ATP} channel records (80 s in duration) in the absence (*top*) and presence (*bottom*) of 1 mM UDP. Records under both conditions are presented on compressed and extended time scales. Zero-current level is indicated by 0 pA in the compressed, 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 τ_o and $\tau_{c,1}$. (*right*) Distribution of openings within a burst in the presence of 1 mM of 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, τ_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.

each of the inhibitory ligands (56 ms, n_{burst} = 255 and 850 ms, n_{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):

$$\sigma_{burst} = \frac{k_{10} + k_{01}}{k_{10}k_{02}} \quad (8)$$

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:

$$\sigma_{C2} = \frac{1}{k_{20} + k_{23}}; \quad \sigma_{C3} = \frac{1}{k_{32}}, \quad (9)$$

the lifetime the K_{ATP} channel spent in C_2 or C_3, in the presence of ATP (100 μM), was 12.2 and 175 ms, respectively. Although these values were within the range of values obtained in the absence of ATP (σ_{C2} = 9.1 ± 1.4 ms, and σ_{C3} = 207 ± 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_{ATP} channel spent within interburst transitions (Sakmann and Trube, 1984):

$$\sigma_{C2,3} = \frac{k_{23} + k_{32}}{k_{32}k_{20}} \quad (10)$$

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

Glyburide (1 μM) 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 ($\sigma_{C2,3}$) 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-

TABLE I

Parameters of Open and Closed Time Distributions of K_{ATP} Channel Activity Obtained under Different Experimental Conditions

	Sustained channel activity								Partial rundown	
	−UDP	+UDP	−ATP	+ATP	−Glyburide	+Glyburide	−UDP	+UDP+ATP	−UDP	+UDP
Number of events within burst (N_{IB})	27115	23094	18022	3825	13022	11255	9113	9471	38094	33183
Number of events between burst (N_B)	27	2	23	255	14	31	14	4	224	125
Gaps within a burst ($\tau_{c,1}$), ms	0.44	0.44	0.49	0.53	0.53	0.54	0.5	0.5	0.53	0.50
Gaps between bursts, fast ($\tau_{c,2}$), ms	5.34	—	9.4	12.1	9.8	21	12	—	11.9	3.05
Gaps between burst, slow ($t_{c,3}$), ms	39.3	—	230	700	544	1500	383	—	842	35.4
Relative area of $\tau_{c,2}$ (a_2)	0.6	—	0.66	0.27	0.77	0.58	0.74	—	0.83	0.52
Relative area of $\tau_{c,3}$ (a_3)	0.4	—	0.34	0.73	0.23	0.42	0.26	—	0.17	0.48
Open time (τ_o), ms	2.83	2.9	2.3	2.2	2.1	2.1	2.3	2.4	1.9	1.9

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 (3 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., 1994*a*; Brady at 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., 1994*a*). 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-

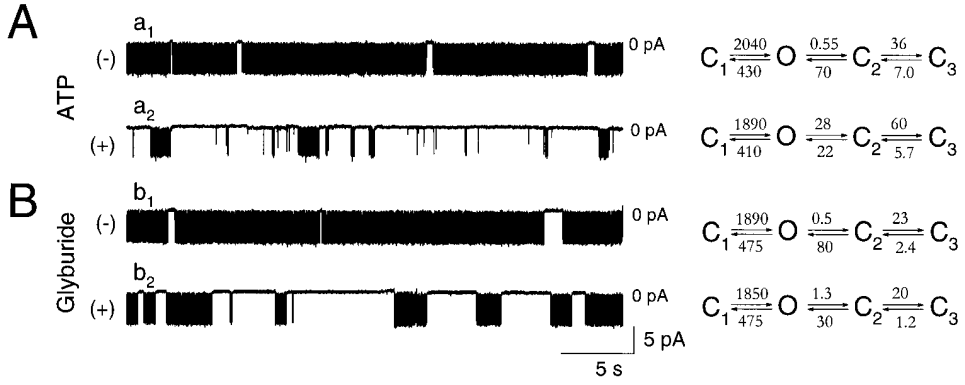

FIGURE 2. Inhibitory ligands act on K_{ATP} channels outside intraburst transition. Portions of original single channel records in the absence (a_1 and b_1) and presence (a_2) of 100 μM ATP or 1 μM glyburide (b_2). Corresponding kinetic schemes with calculated rates of transitions (in s^{-1}, Eq. 8) 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.

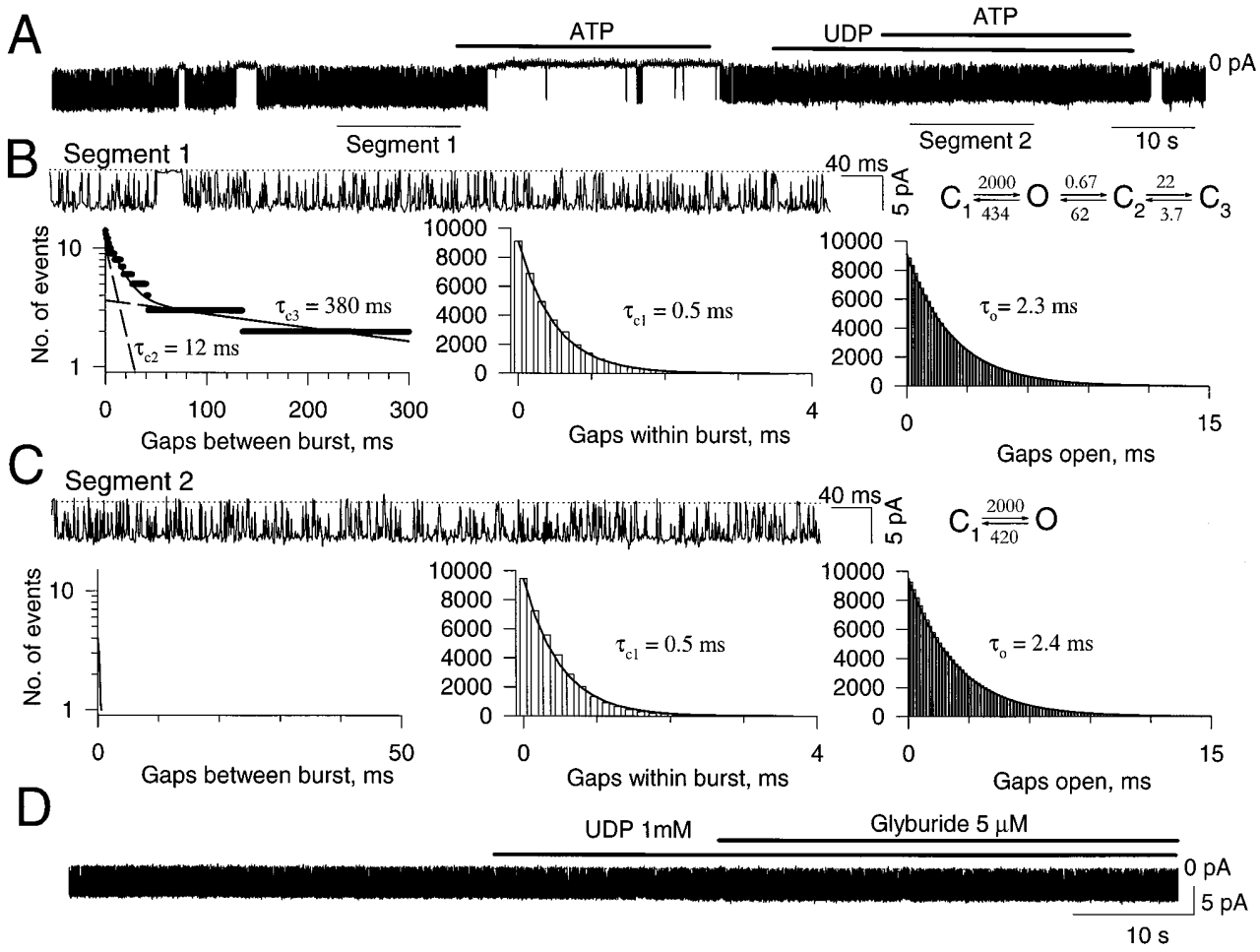

FIGURE 3. Loss of interburst events induced by UDP associated with loss of ATP or glyburide sensitivity of K_{ATP} channels. (*A*) Single-channel record of K_{ATP} channel activity inhibited by ATP (200 μM) in the absence but not in the presence of UDP (1 mM). (*B*) Single-channel record of segment 1 (in *A*) presented on an extended time scale with distributions for gaps between burst (*left*), gaps within burst (*middle*), and open gaps (*right*). Corresponding kinetic scheme with rates of transitions (s^{-1}, Eq. 8) is provided. (*C*) Single-channel record of segment 2 (in *A*), obtained in the presence of UDP (1 mM) and ATP (200 μM) and presented on an extended time scale with distributions for gaps between burst (*left*), gaps within burst (*middle*), and open gaps (*right*). In the presence of both UDP and ATP, essentially no gaps between bursts were visible, while no effect on intraburst kinetics was observed. A corresponding kinetic scheme, without interburst transitions and with calculated rates of intraburst transitions (s^{-1}), is provided. (*D*) Single-channel record of K_{ATP} channel activity depicting UDP-induced antagonism of glyburide-mediated channel inhibition. 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_{ATP} Channels within Intraburst Transitions

Progression of rundown of K_{ATP} channel activity was associated with an increased number of prolonged closures (Fig. 5 *A*, *top*). Intraburst channel properties ($\tau_{c,1}$ and τ_0, Fig. 5 *B*, Table I) were unchanged with rundown, but both fast and slow time components defining gaps between burst ($\tau_{c,2}$ and $\tau_{c,3}$) were prolonged (Fig. 5 *C*, Table I). As the process of rundown accelerated, the k_{02} rate responsible for exit of the channel from a burst (from 0.52 ± 0.07 s^{-1}, n = 4, under sustained activity to 3.1 s^{-1} during rundown; Fig. 5), mean burst duration of partial rundown channels was shorter when compared with sustained channel activity (τ_{burst} = 440 ms; n_{burst} = 224 vs. τ_{burst} = 2,456 ± 315 ms; n = 4). Furthermore, K_{ATP} channels under partial rundown spent a significantly longer time in the C_3 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_2 state (12 ms vs. 9.1 ± 1.4 ms, n = 4; Eq. 10) was similar under both conditions. Thus, channel rundown directed K_{ATP} channels away from intraburst and towards interburst transitions.

To determine the efficacy with which UDP acts on K_{ATP} channels driven towards the C_3 state as rundown progresses, UDP (1 mM) was applied to partially run-

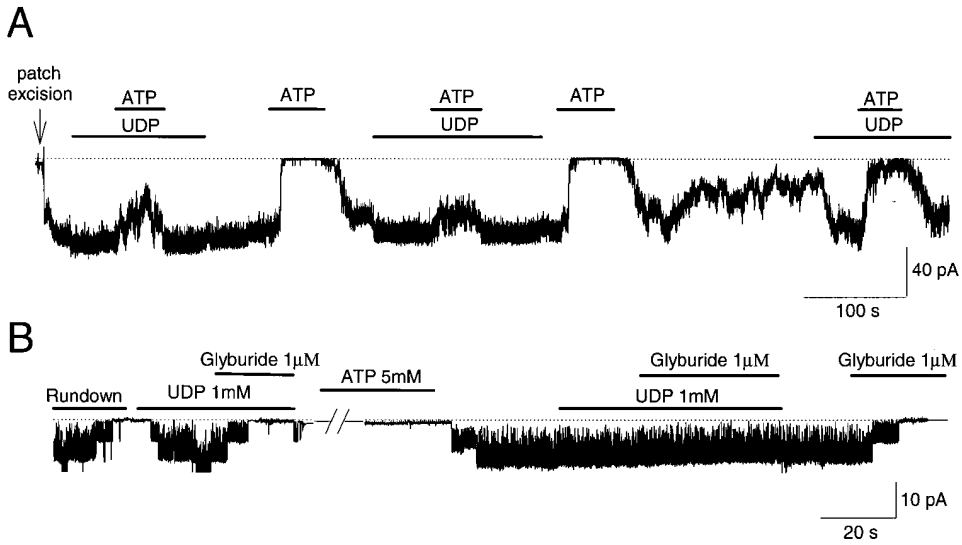

FIGURE 4. Dual responsive behavior of K_{ATP} channels, in the presence of UDP, towards ATP and glyburide. (*A*) After patch excision, K_{ATP} channel activity was vigorous and sustained at maximal level by UDP (2 mM), under which condition ATP (300 μM) produced only partial channel inhibition. Removal of UDP was associated with channel inhibition by ATP. Such effect was reproducible. With time after patch excision, K_{ATP} channel activity was observed to rundown. Under partial rundown, UDP enhanced channel activity, but only partially antagonized ATP-induced channel inhibition. The dotted line corresponds to the zero-current level. (*B*) Conversion of rundown to spontaneous K_{ATP} channel activity by Mg-ATP switches on the UDP-induced antagonism of glyburide-dependent channel block. A 10-min long pretreatment of rundown K_{ATP} 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. nP_o values, corresponding to the trace record, were calculated over 1.02-s-long intervals. Holding potential was −60 mV throughout.

down channels (Fig. 5 *A*, *bottom*). UDP increased channel activity and reduced the number of channel closures (Fig. 5 *A*, *bottom*). Similar to its effect on sustained K_{ATP} channel activity, UDP did not affect intraburst kinetics; i.e., UDP did not significantly change rates defining the $C_1 \leftrightarrow O$ transition (k_{10} and k_{01}) of partially rundown channels (Fig. 5, *A* and *B*). However, in contrast to sustained K_{ATP} channel activity (Fig. 1), UDP did not eliminate interburst events in rundown channels, the distribution of which remained biexponential (Fig. 5 *C*). Also, in contrast to its effect on sustained channel activity where UDP eliminated the $O \leftrightarrow C_2$ transition (from 0.35 to ~0 s^{-1}; Fig. 1, Table I), UDP only decreased the transition rate (k_{02}) associated with burst closure of partially rundown channels (i.e., the $O \leftrightarrow C_2$ transition changed from 3.1 to 2.0 s^{-1}; Fig. 5 *D*). Under this condition, the K_{ATP} channel could still transit between intraburst and interburst states despite the presence of UDP (Fig. 5, *A* and *C*). Mean burst duration (Eq. 9) of partially rundown channels increased in the presence of UDP from τ_{burst} = 440 ms (n_{burst} = 224) to τ_{burst} = 684 ms (n_{burst} = 125), similar to calculated values of τ_{burst} (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_2 and C_3, respectively; Fig. 5 *A*; 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., 1994*a*), whereas it completely eliminates the sensitivity of the K_{ATP} channel to glyburide (Brady et al., 1998). Based on the kinetic model, it was not possible to quantitatively predict the different response of K_{ATP} 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 ($K_{A,S}$ or $K_{B,S}$ in *S* and $K_{A,I}$ or $K_{B,I}$ 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-

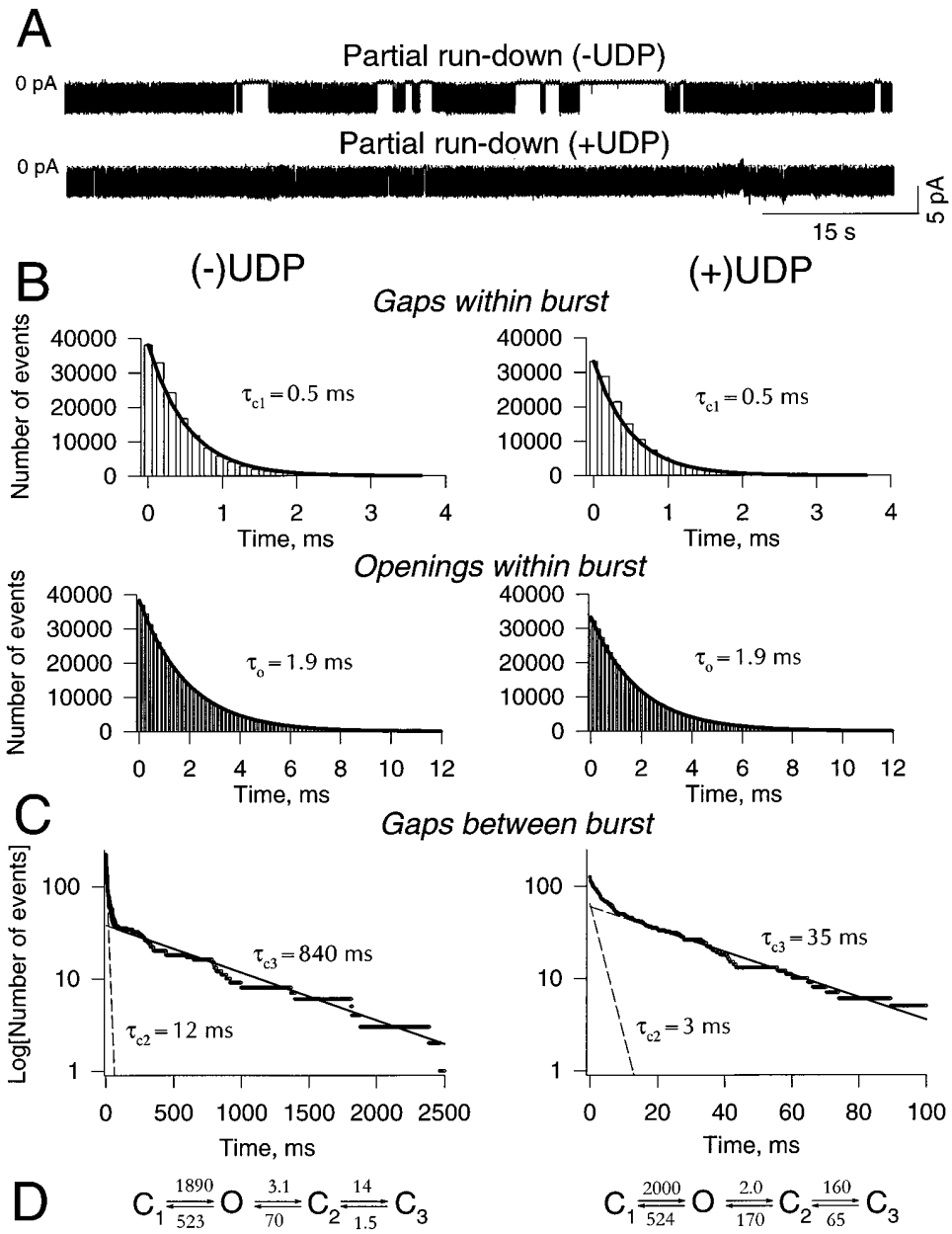

FIGURE 5. Rundown prevents UDP from locking K_{ATP} channels within intraburst transitions. (*A*) Single K_{ATP} channel records during partial rundown in the absence (*top*) and presence (*bottom*) of 1 mM UDP. Zero-current level indicated by 0 pA. (*B*) Intraburst kinetic properties. UDP (*right*) did not affect distributions of closed (*top*) and open (*bottom*) times within bursts of channel activity. Both distributions were well-fitted by single exponents, with $\tau_{c,1}$ and τ_o representing characteristic closed and open times, respectively. (*C*) Distribution of gaps between bursts in the absence (*left*) and presence (*right*) of UDP. Under both conditions, distributions needed to be fitted by the sum of two exponents with characteristic times $\tau_{c,2}$ and $\tau_{c,3}$. Solid lines correspond to the sum of both exponents drawn by fitting, whereas dashed lines correspond to individual exponents. Holding potential was −60 mV. (*D*) Kinetic scheme constructed based on calculated rates of transitions (in s^{-1}, Eq. 8) in the absence (*left*) and presence (*right*) of 1 mM UDP.

nel 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:

$$L = \frac{\sigma_{burst}}{\sigma_{C2,3}} = \frac{(k_{10} + k_{01})\, k_{32} k_{20}}{(k_{23} + k_{32})\, k_{10} k_{02}}. \qquad (11)$$

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:

$$\bar{S} = \frac{1}{1 + L\left[\frac{1 + d\beta}{1 + \beta}\right]^m \left[\frac{1 + c\alpha}{1 + \alpha}\right]^n} \qquad (12)$$

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

In the absence of UDP, the concentration dependence of K_{ATP} channel inhibition by glyburide (*A*; Fig. 6

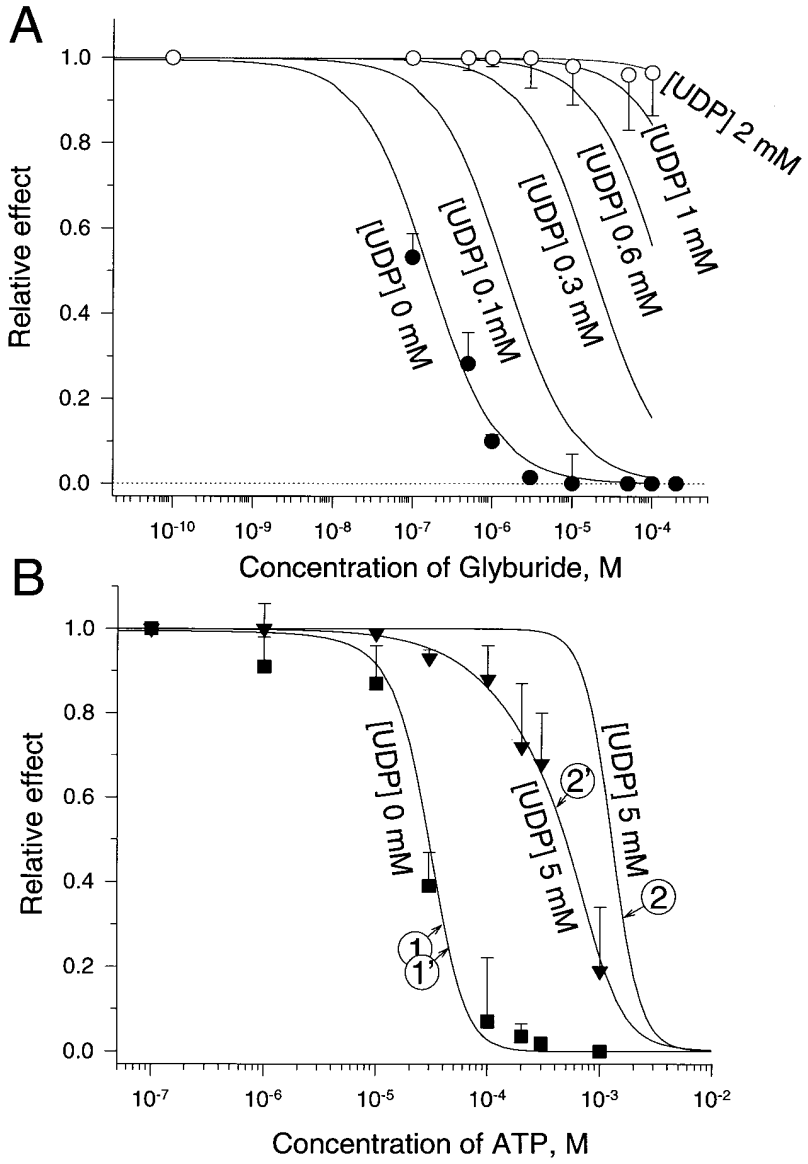

FIGURE 6. Use of an allosteric model to predict the effect of glyburide or ATP on K_{ATP} channel opening in the absence and presence of UDP. (*A*) Concentration dependence of glyburide-induced K_{ATP} channel inhibition in the absence (●) and presence (○) of 1 mM UDP. Data points are from five to nine patches. Relative effect of glyburide was calculated in each patch as a ratio between slopes of cumulative nP_o measured in the presence over the value obtained in the absence of glyburide (see Brady et al., 1998). Solid curves were constructed using Eq. 13 at various concentrations of UDP (see text for values of parameters). (*B*) Concentration dependence of ATP-induced K_{ATP} channel inhibition in the absence (■) and presence (▼) of 5 mM UDP. Data points are from 4 to 10 patches. Relative effect of ATP was calculated in each patch as a ratio between nP_o values measured in the presence over the value obtained in the absence of ATP (see Terzic et al., 1994*a*). Curves 1 and 1′ (at 0 mM UDP), 2 and 2′ (at 5 mM UDP) were constructed using Eqs. 13 and 14, respectively. See text for values of parameters.

A, ●) was well fitted by the $1\text{-}\bar{S}$ function (Eq. 13) using the following parameters: $L = 200$; $K_{A,S} = 1$ nM, $K_{A,I} = 1$ mM, $n = 1$ (Fig. 6 *A, solid line*). In the presence of 1 mM UDP, the observed loss of sensitivity of the K_{ATP} channel towards the sulfonylurea (Fig. 6 *A*, ○) was well described by the allosteric model ($L = 200$; $K_{A,S} = 1$ nM, $K_{A,I} = 1$ mM, $n = 1$; $K_{B,S} = 3.5$ mM, $K_{B,I} = 0.1$ mM, $m = 4$). The model predicts that at millimolar concentrations of the activator (UDP) the effect of glyburide on K_{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_{ATP} channel inhibition by ATP (*A*; Fig. 6 *B*, ■) was also well fitted by the $1\text{-}\bar{S}$ function (Eq. 13) using the following parameters: $L = 200$; $K_{A,S} = 8.5$ μM, $K_{A,I} = 70$ mM, $n = 3.6$ (Fig. 6 *B*, curve *1*). However, in the presence of UDP (5 mM; $K_{B,S} = 3.5$ mM, $K_{B,I} = 0.1$ 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_{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 $1\text{-}\bar{S}$ function (Eq. 13) an additional allosteric regulation-independent inhibitory process to account for both mechanisms of ATP inhibitory action:

$$[\text{Relative effect}] = \{1 - \overline{S}\} \cdot \frac{K_d}{K_d + [A]} \quad (13)$$

where K_d is the dissociation constant to this additional ATP binding site. In this adjusted model, $K_d = 610$ μM could fully account for the ATP-induced channel inhibition in the absence (Fig. 6 *B*, curve *1′*) and presence (Fig. 6 *B*, curve *2′*) of UDP. The prediction based on the developed model can be interpreted to mean that UDP antagonized ATP-induced K_{ATP} channel inhibition through one set of ATP-binding sites, thus reducing channel inhibition to the other set of ATP-binding sites.

Despite the lack of reliable means to quantify the rundown process, the allosteric model could, in principle, also be used to describe the effects of ligands under rundown of K_{ATP} channel activity. Based on our experimental data (Fig. 5), rundown shifted the equilibrium constant (L) from 150–200 under spontaneous channel activity to 10–20 under partial rundown, and to even lower values with further progression of rundown. This was associated with a prolongation of the lifetime the channel spent in C_2 and C_3 closed states. In contrast to spontaneous channel activity, where UDP increased $L \rightarrow \infty$(by $k_{02} \rightarrow 0$), 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*a*; 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_{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_{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_{ATP} channel regulation. The property of a nucleotide diphosphate to direct cardiac K_{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_{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_{ATP} channel current as recorded under present experimental conditions (with symmetrical K^+ solutions and at a holding potential of −60 mV), intraburst and interburst transitions can also be distinguished for outward K_{ATP} channel currents despite more complex intraburst kinetics (Zilberter et al., 1988; Larsson et al., 1993; Alekseev et al., 1997*b*). Herein, we found that the inhibitory ligands, ATP and glyburide, inhibited K_{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_{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., 1994*a*; Brady et al., 1998). Based on the kinetic model used, the present study provides evidence that the response of the cardiac K_{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., 1994*b*), intracellular protons (Vivaudou and Forestier, 1995; Alekseev et al., 1997*a*), cytoskeleton disrupters (Brady et al., 1996*a*), or channel trypsinization (Deutsch and Weiss, 1994), which also promote the K_{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_{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_{ATP} channel to operate within interburst transitions were shown to enhance the sensitivity of K_{ATP} channels towards sul-

fonylureas (Virag et al., 1993; Schwanstecher et al., 1994).

Application of an Allosteric Model to the Regulation of K_{ATP} Channels

The dual nature of K_{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_{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_{ATP} channel loses its sensitivity towards glyburide. This is in accord with previous studies that have established that under spontaneous K_{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_{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., 1994*a*) 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_{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_{ATP} channel activity (Thuringer and Escande, 1989; Deutsch and Weiss, 1993). Since rundown is believed to be associated with changes in the phosphorylation status of the K_{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; Hilgemann 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 "rephosphorylation" of channel proteins, restored spontaneous cardiac K_{ATP} channel activity, and with it the efficacy of UDP to antagonize ATP- and glyburide-induced channel inhibition (see also Terzic et al., 1994*a*; Brady et al., 1998).

Relevance to the Molecular Structure of K_{ATP} Channels

Results predicted by the allosteric model applied herein to the native cardiac K_{ATP} channel are in agreement with the reported structure and stoichiometry of the recombinant K_{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_{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_{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_{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., 1997*b*; 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_{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_{ATP} channel gating (Fig. 7). Such a model implies the existence of two inhibitory gating pathways. The first, mediated through binding of inhibi-

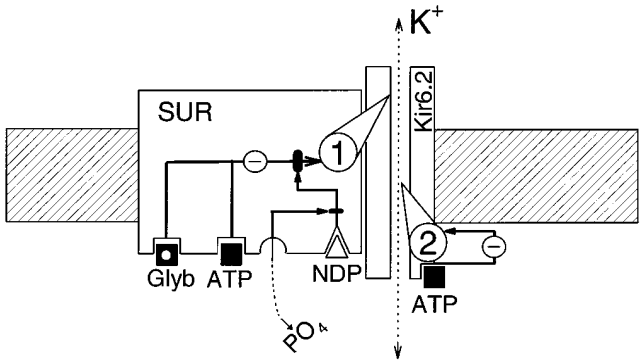

FIGURE 7. Scheme of UDP-induced change in ATP- and glyburide-dependent inhibitory channel gating. This mechanistic model, which takes into account the proposed structure of the K_{ATP} channel (Inagaki et al., 1995, 1996, 1997; Clement et al., 1997; Tucker et al., 1997), as well as the kinetic and allosteric properties of channel behavior, suggests the existence of two inhibitory gating mechanisms of K_{ATP} channels labeled 1 and 2. The gating mechanism number 1 transduces inhibitory signals from glyburide (*Glyb*) and ATP-binding sites on the SUR channel subunit. This inhibitory gating can be intercepted after binding of a nucleotide-diphosphate (*NDP*) to the SUR subunit. Presumed dephosphorylation of the channel affects nucleotide diphosphate–dependent regulation of channel gating. The gating mechanism number 2 transduces inhibitory signals from the ATP-binding site on the Kir6.2 channel subunit, which appears to be insensitive to nucleotide-diphosphate regulation.

tory 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_{ATP} 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_{ATP} 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_{ATP} 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^+ and Ca^{2+} channels (Lee and Tsien, 1983; Hill et al., 1989; Ragsdale at al., 1994; Nuss et al., 1995). Entry of the K_{ATP} channel into a ligand-insensitive state by a nucleotide diphosphate could provide a basis for cardiac K_{ATP} channel opening during hypoxia or ischemia despite rather constant levels of ATP within the cell.

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REFERENCES

Aguilar-Bryan, L., C.G. Nichols, A.S. Rajan, C. Parker, and J. Bryan. 1992. Co-expression of sulfonylurea receptors and K_{ATP} channels in hamster insulinoma tumor (HIT) cells. Evidence for direct association of the receptor with the channel. *J. Biol. Chem.* 267: 14934–14940.

Aguilar-Bryan, L., C.G. Nichols, S.W. Wechsler, J.P. Clement IV, A.E. Boyd III, G. Gonzalez, H. Herrera-Sosa, K. Nguy, J. Bryan, and D.A. Nelson. 1995. Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science.* 268: 423–426.

Alekseev, A.E., L.A. Gomez, L.A. Aleksandrova, P.A. Brady, and A. Terzic. 1997*a*. Opening of cardiac sarcolemmal K_{ATP} channels by dinitrophenol separate from metabolic inhibition. *J. Membr. Biol.* 157:203–214.

Alekseev, A.E., M.E. Kennedy, B. Navarro, and A. Terzic. 1997*b*. Burst kinetics of co-expressed Kir6.2/SUR1 clones: comparison of recombinant with native ATP-sensitive K^+ channel behavior. *J. Membr. Biol.* 159:161–168.

Alekseev, A.E., A. Jovanovic, J.R. Lopez, and A. Terzic. 1996*a*. Adenosine slows the rate of K^+-induced membrane depolarization in ventricular cardiomyocytes: possible implication in hyperkalemic cardioplegia. *J. Mol. Cell. Cardiol.* 28:1193–1202.

Alekseev, A.E., N.I. Markevich, A.F. Korystova, A. Terzic, and Y.M. Kokoz. 1996*b*. Comparative analysis of the kinetic characteristics

of L-type calcium channels in cardiac cells of hibernators. *Biophys. J.* 70:786–797.

Allard, B., and M. Lazdunski. 1992. Nucleotide diphosphates activate the ATP-sensitive potassium channel in mouse skeletal muscle. *Pflügers Arch. Eur. J. Physiol.* 422:185–192.

Aschroft, F.M., and S.J.H. Ashcroft. 1990. Properties and functions of ATP-sensitive K^+ channels. *Cell. Signal.* 2:197–214.

Benz, I., and M. Kohlhardt. 1994. Distinct modes of blockade in cardiac ATP-sensitive K^+ channels suggest multiple targets for inhibitory drug molecules. *J. Membr. Biol.* 142:309–322.

Bernardi, H., M. Fosset, and M. Lazdunski. 1992. ATP/ADP binding sites are present in the sulfonylurea binding protein associated with brain ATP-sensitive K^+ channels. *Biochemistry.* 31:6328–6332.

Bokvist, K., C. Ämmälä, F.M. Ashcroft, P.-O. Berggren, O. Larsson, and P. Rorsman. 1991. Separate processes mediate nucleotide-induced inhibition and stimulation of the ATP-regulated K^+-channels in mouse pancreatic β-cells. *Proc. R Soc. Lond. Ser. B. Biol. Sci.* 243:139–144.

Brady, P.A., A.E. Alekseev, L.A. Aleksandrova, L.A. Gomez, and A. Terzic. 1996*a*. A disrupter of actin microfilaments impairs sulfonylurea-inhibitory gating of cardiac K_{ATP} channels. *Am. J. Physiol.* 271:H2710–H2716.

Brady, P.A., A.E. Alekseev, and A. Terzic. 1998. Operative condition-dependent response of cardiac ATP-sensitive K^+ channels toward sulfonylureas. *Circ. Res.* In press.

Brady, P.A., S. Zhang, J.R. Lopez, A. Jovanovic, A.E. Alekseev, and A. Terzic. 1996*b*. Dual effect of glyburide, an antagonist of K_{ATP} channel, on metabolic inhibition-induced Ca^{2+} loading in cardiomyocytes. *Eur. J. Pharmacol.* 308:343–349.

Bryan, J., and L. Aguilar-Bryan. 1997. The ABCs of ATP-sensitive potassium channels—more pieces of the puzzle. *Curr. Opin. Cell Biol.* 9:553–559.

Clement, J.P., K. Kunjilwar, G. Gonzalez, M. Schwanstecher, U. Panten, L. Aguilar-Bryan, and J. Bryan. 1997. Association and stoichiometry of K-ATP channel subunits. *Neuron.* 18:827–838.

Decking, U.K.M., T. Reffelmann, J. Schrader, and H. Kammermeier. 1995. Hypoxia-induced activation of K-ATP channels limits energy depletion in the guinea pig heart. *Am. J. Physiol.* 269: H734–H742.

Decking, U.K.M., G. Schlieper, K. Kroll, and J. Schrader. 1997. Hypoxia-induced inhibition of adenosine kinase potentiates cardiac adenosine release. *Circ. Res.* 81:154–164.

Deutsch, N., and J.N. Weiss. 1993. ATP-sensitive K^+ channel modification by metabolic inhibition in isolated guinea-pig ventricular myocytes. *J. Physiol.* 465:163–179.

Deutsch, N., and J.N. Weiss. 1994. Effects of trypsin on cardiac ATP-sensitive K^+ channels. *Am. J. Physiol.* 266:H613–H622.

Dunne, M.J., and O.H. Petersen. 1986. Intracellular ADP activates K^+ channels that are inhibited by ATP in an insulin-secreting cell line. *FEBS Lett.* 208:58–62.

Elvir-Mairena, J.R., A. Jovanovic, L.A. Gomez, A.E. Alekseev, and A. Terzic. 1996. Reversal of the ATP-liganded state of ATP-sensitive K^+ channels by adenylate kinase. *J. Biol. Chem.* 271:31903–31908.

Fan, Z., K. Nakayama, and M. Hiraoka. 1990. Multiple actions of pinacidil on adenosine triphosphate-sensitive potassium channels in guinea-pig ventricular myocytes. *J. Physiol.* 430:273–295.

Findlay, I. 1987. The effects of magnesium upon adenosine trisphosphate-sensitive potassium channels in a rat insulin-secreting cell line. *J. Physiol.* 391:611–629.

Findlay, I. 1988. Effects of ADP upon the ATP-sensitive K^+ channel in rat ventricular myocytes. *J. Membr. Biol.* 101:83–92.

Findlay, I. 1994. Interactive regulation of the ATP-sensitive potassium channel of cardiac muscle. *J. Cardiovasc. Pharmacol.* 24:S6–S11.

Findlay, I., and M.J. Dunne. 1986. ATP maintains ATP-inhibited K^+ channels in an operational state. *Pflügers Arch. Eur. J. Physiol.* 407: 238–240.

Forestier, C., and M. Vivaudou. 1993. Modulation by Mg^{2+} and ADP of ATP-sensitive potassium channels in frog skeletal muscle. *J. Membr. Biol.* 132:87–94.

Fosset, M., J.R. De Weille, R.D. Green, H. Schmid-Antomarchi, and M. Lazdunski. 1988. Antidiabetic sulfonylureas control action potential properties in heart cells via high affinity receptors that are linked to ATP-dependent K^+ channels. *J. Biol. Chem.* 263:7933–7936.

Furukawa, T., L. Virag, T. Sawanobori, and M. Hiraoka. 1993. Stilbene disulfonates block ATP-sensitive K^+ channels in guinea pig ventricular myocytes. *J. Membr. Biol.* 136:289–302.

Furukawa, T., T. Yamane, T. Terai, Y. Katayama, and M. Hiraoka. 1996. Functional linkage of the cardiac ATP-sensitive K^+ channel to the actin cytoskeleton. *Pflugers Archiv. Eur. J. Physiol.* 431:504–512.

Gillis, K.D., W.M. Gee, A. Hammoud, M.L. McDaniel, L.C. Falke, and S. Misler. 1989. Effects of sulfonamides on a metabolite-regulated ATP_i-sensitive K^+ channel in rat pancreatic β-cells. *Am. J. Physiol.* 257:C1119–C1127.

Gribble, F.M., S.J. Tucker, and F.M. Ashcroft. 1997. The essential role of the walker a motifs of SUR1 in K_{ATP} channel activation by Mg-ADP and diazoxide. *EMBO (Eur. Mol. Biol. Organ.) J.* 16:1145–1152.

Hill, R.J., H.J. Duff, and R.S. Sheldon. 1989. Class I antiarrhythmic drug receptor: biochemical evidence for state-dependent interaction with quinidine and lidocaine. *Mol. Pharmacol.* 36:150–159.

Inagaki, N., T. Gonoi, J.P. Clement IV, N. Namba, J. Inazawa, G. Gonzalez, L. Aguilar-Bryan, S. Seino, and J. Bryan. 1995. Reconstitution of I_{KATP}: an inward rectifier subunit plus the sulfonylurea receptor. *Science.* 270:1166–1170.

Inagaki, N., T. Gonoi, J.P. Clement IV, C.Z. Wang, L. Aguilar-Bryan, J. Bryan, and S. Seino. 1996. A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels. *Neuron.* 16:1011–1017.

Inagaki, N., T. Gonoi, and S. Seino. 1997. Subunit stoichiometry of the pancreatic beta-cell ATP-sensitive K^+ channel. *FEBS Lett.* 409: 232–236.

Isomoto, S., C. Kondo, M. Yamada, S. Matsumoto, O. Higashiguchi, Y. Horio, Y. Matsuzawa, and Y. Kurachi. 1996. A novel sulfonylurea receptor forms with BIR (Kir6.2) a smooth muscle type ATP-sensitive K^+ channel. *J. Biol. Chem.* 271:24321–24324.

Jovanovic, A., S. Zhang, A.E. Alekseev, and A. Terzic. 1996. Diadenosine polyphosphate-induced inhibition of cardiac K_{ATP} channels: operative state-dependent regulation by a nucleoside diphosphate. *Pflügers Arch. Eur. J. Physiol.* 431:800–802.

Jovanovic, A., A.E. Alekseev, and A. Terzic. 1997. Intracellular diadenosine polyphosphates: a novel family of inhibitory ligands of the ATP-sensitive K^+ channel. *Biochem. Pharmacol.* 54:219–225.

Hilgemann, D.W., and R. Ball. 1996. Regulation of cardiac Na^+, Ca^{2+} exchange and K_{ATP} potassium channels by PIP_2. *Science.* 273: 956–959.

Hosoya, Y., M. Yamada, H. Ito, and Y. Kurachi. 1996. A functional model for G protein activation of the muscarinic K^+ channel in guinea pig atrial myocytes. Spectral analysis of the effect of GTP on single-channel kinetics. *J. Gen. Physiol.* 108:485–495.

Kakei, M., and A. Noma. 1984. Adenosine-5′-triphosphate-sensitive single potassium channel in the atrioventricular node cell of the rabbit heart. *J. Physiol.* 352:265–284.

Kakei, M., R.P. Kelly, S.J.H. Ashcroft, and F.M. Ashcroft. 1986. The ATP-sensitivity of K^+ channels in pancreatic β-cells is modulated by ADP. *FEBS Lett.* 208:63–66.

Karlin, A. 1967. On the application of "a plausible model" of allosteric proteins to the receptor for acetylcholine. *J. Theor. Biol.* 16: 306–320.

Larsson, O., C. Ammala, K. Bokvist, B. Fredholm, and P. Rorsman. 1993. Stimulation of the K_{ATP} channel by ADP and diazoxide requires nucleotide hydrolysis in mouse pancreatic β-cells. *J. Physiol.* 463:349–365.

Lazdunski, M. 1994. ATP-sensitive potassium channels: an overview. *J. Cardiovasc. Pharmacol.* 24:S1–S5.

Lederer, W.J., and C.J. Nichols. 1989. Nucleotide modulation of the activity of rat heart ATP-sensitive K^+ channels in isolated membrane patches. *J. Physiol.* 419:193–211.

Lee, K.S., and R.W. Tsien. 1983. Mechanism of calcium channel blockade by verapamil, D600, diltiazem and nitrendipine in single dialyzed heart cells. *Nature.* 302:790–794.

Misler, S., L.C. Falke, K. Gillis, and M.L. McDaniel. 1986. A metabolite-regulated potassium channel in rat pancreatic β cells. *Proc. Natl. Acad. Sci. USA.* 83:7119–7123.

Monod, J., J. Wyman, and J.-P. Changeux. 1965. On the nature of allosteric transitions: a plausible model. *J. Mol. Biol.* 12:88–118.

Nichols, C.G., and W.J. Lederer. 1991. Adenosine triphosphate-sensitive potassium channels in the cardiovascular system. *Am. J. Physiol.* 261:H1675–H1686.

Nichols, C.G., W.J. Lederer, and M.B. Cannel. 1991. ATP dependence of K_{ATP} channel kinetics in isolated membrane patches from rat ventricle. *Biophys. J.* 60:1164–1177.

Nichols, C.G., S.L. Shyng, A. Nestorowicz, B. Glaser, J.P. Clement IV, G. Gonzalez, L. Aguilar-Bryan, M.A. Permutt, and J. Bryan. 1996. Adenosine diphosphate as an intracellular regulator of insulin secretion. *Science.* 272:1785–1787.

Noma, A. 1983. ATP-regulated K^+ channels in cardiac muscle. *Nature.* 305:147–148.

Nuss, H.B., G.F. Tomaselli, and E. Marban. 1995. Cardiac sodium channels (hH1) are intrinsically more sensitive to block by lidocaine than are skeletal muscle (μ1) channels. *J. Gen. Physiol.* 106:1193–1209.

Ohno-Shosaku, T., B.J. Zünkler, and G. Trube. 1987. Dual effects of ATP on K^+ currents of mouse pancreatic β-cells. *Pflügers Arch. Eur. J. Physiol.* 408:133–138.

Qin, D., M. Takano, and A. Noma. 1989. Kinetics of the ATP-sensitive K^+ channel revealed with oil-gate concentration jump method. *Am. J. Physiol.* 257:H1624–H1633.

Ragsdale, D.S., J.C. McPhee, T. Scheuer, and W.A. Catterall. 1994. Molecular determinants of state-dependent block of Na^+ channels by local anesthetics. *Science.* 265:1724–1728.

Sakmann, B., and G. Trube. 1984. Voltage-dependent inactivation of inward-rectifying single-channel currents in the guinea-pig heart cell membrane. *J. Physiol. (Camb.).* 347:659–683.

Schwanstecher, C., C. Dickel, and U. Panten. 1994. ATP-sensitive K^+ channel in mouse beta-cells. *Br. J. Pharmacol.* 111:302–310.

Seino, S., N. Inagaki, N. Namba, and T. Gonoi. 1996. Molecular biology of the β-cell ATP-sensitive K^+ channel. *Diabetes Rev.* 4:177–190.

Smith, P.A., B.A. Williams, and F.M. Ashcroft. 1994. Block of ATP-sensitive K^+ channels in isolated mouse pancreatic beta-cells by 2,3-butanedione monoxime. *Br. J. Pharmacol.* 112:143–149.

Takano, M., and A. Noma. 1993. The ATP-sensitive K^+ channel. *Prog. Neurobiol.* 41:21–30.

Takano, M., D. Qin, and A. Noma. 1990. ATP-dependent decay and recovery of K^+ channels in guinea-pig cardiac myocytes. *Am. J. Physiol.* 258:H45–H50.

Terzic, A., I. Findlay, Y. Hosoya, and Y. Kurachi. 1994*a*. Dualistic behavior of ATP-dependent K^+ channel towards intracellular nucleotide diphosphates. *Neuron.* 12:1049–1058.

Terzic, A., A. Jahangir, and Y. Kurachi. 1994*b*. HOE-234, a second generation K^+ channel opener, antagonizes the ATP-dependent gating of cardiac ATP-sensitive K^+ channels. *J. Pharmacol. Exper. Ther.* 68:818–825.

Terzic, A., A. Jahangir, and Y. Kurachi. 1995. Cardiac ATP-sensitive K^+ channels: regulation by intracellular nucleotides and K^+ channel-opening drugs. *Am. J. Physiol.* 269:C525–C545.

Terzic, A., and Y. Kurachi. 1996. Actin microfilament disrupters enhance K_{ATP} channel opening in patches from guinea-pig cardiomyocytes. *J. Physiol. (Camb.).* 492:395–404.

Terzic, A., R. Tung, A. Inanobe, T. Katada, and Y. Kurachi. 1994*c*. G proteins activate ATP-sensitive K^+ channels by antagonizing ATP-dependent gating. *Neuron.* 12:885–893.

Terzic, A., R. Tung, and Y. Kurachi. 1994*d*. Nucleotide regulation of ATP-sensitive K^+ channels. *Cardiovasc. Res.* 28:746–753.

Tibbs, G.R., E.H. Goulding, and S.A. Siegelbaum. 1997. Allosteric activation and tuning of ligand efficacy in cyclic-nucleotide-gated channels. *Nature.* 386:612–615.

Thuringer, D., and D. Escande. 1989. Apparent competition between ATP and the potassium channel opener RP-49356 on ATP-sensitive K^+ channels of cardiac myocytes. *Mol. Pharmacol.* 36: 897–902.

Trapp, S., S.J. Tucker, and F.M. Ashcroft. 1997. Activation and inhibition of K-ATP currents by guanine nucleotides is mediated by different channel subunits. *Proc. Natl. Acad. Sci. USA.* 94:8872–8877.

Trube, G., and J. Hescheler. 1984. Inward-rectifying channels in isolated patches of the heart cell membrane: ATP-dependence and comparison with cell-attached patches. *Pflügers Arch. Eur. J. Physiol.* 401:178–184.

Tucker, S., F.M. Gribble, C. Zhao, S. Trapp, and F.M. Ashcroft. 1997. Truncation of Kir6.2 produces ATP-sensitive K^+ channels in the absence of the sulphonylurea receptor. *Nature.* 387:179–183.

Tung, R.T., and Y. Kurachi. 1991. On the mechanism of nucleotide diphosphate activation of the ATP-sensitive K^+ channel in ventricular cell of guinea-pig. *J. Physiol. (Camb.).* 437:239–256.

Ueda, K., N. Inagaki, and S. Seino. 1997. MgADP antagonism to Mg^{2+}-independent ATP binding of the sulfonylurea receptor SUR1. *J. Biol. Chem.* 272:22983–22986.

Venkatesh, N., S.T. Lamp, and J.N. Weiss. 1991. Sulfonylureas, ATP-sensitive K^+ channels, and cellular K^+ loss during hypoxia, ischemia and metabolic inhibition in mammalian ventricle. *Circ. Res.* 69:623–637.

Virag, L., T. Furukawa, and M. Hiraoka. 1993. Modulation of the effect of glibenclamide on K_{ATP} channels by ATP and ADP. *Mol. Cell. Biochem.* 119:209–215.

Vivaudou, M., and C. Forestier. 1995. Modification by protons of frog skeletal muscle K_{ATP} channels: effect on ion conduction and nucleotide inhibition. *J. Physiol. (Camb.).* 486:629–645.

Weiss, J., and B. Hiltbrand. 1985. Functional compartmentation of glycolytic versus oxidative metabolism in isolated rabbit heart. *J. Clin. Invest.* 75:436–447.

Weiss, J.N., and N. Venkatesh. 1993. Metabolic regulation of cardiac ATP-sensitive K^+ channels. *Cardiovasc. Drugs Ther.* 7:499–505.

Zilberter, Y., N. Burnashev, A. Papin, V. Portnov, and B. Hodorov. 1988. Gating kinetics of ATP-sensitive single potassium channels in myocardial cells depends on electromotive force. *Pflügers Arch. Eur. J. Physiol.* 411:584–589.