J. Membrane Biol. 159, 161–168 (1997)

The Journal of
Membrane
Biology

Burst Kinetics of Co-expressed Kir6.2/SUR1 Clones: Comparison of Recombinant with Native ATP-sensitive K^+ Channel Behavior

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Received: 12 March 1997/Revised: 5 May 1997

Abstract. Co-expression of clones encoding Kir6.2, a K^+ inward rectifier, and SUR1, a sulfonylurea receptor, reconstitutes elementary features of ATP-sensitive K^+ (K_{ATP}) channels. However, the precise kinetic properties of Kir6.2/SUR1 clones remain unknown. Herein, intraburst kinetics of Kir6.2/SUR1 channel activity, heterologously co-expressed in COS cells, displayed mean closed times from 0.7 ± 0.1 to 0.4 ± 0.03 msec, and from 0.4 ± 0.1 to 2.0 ± 0.2 msec, and mean open times from 1.9 ± 0.4 to 4.5 ± 0.8 msec, and from 12.1 ± 2.4 to 5.0 ± 0.2 msec between −100 and −20 mV, and +20 to +80 mV, respectively. Burst duration for Kir6.2/SUR1 activity was 17.9 ± 1.8 msec with 5.6 ± 1.5 closings *per* burst. Burst kinetics of the Kir6.2/SUR1 activity could be fitted by a four-state kinetic model defining transitions between one open and three closed states with forward and backward rate constants of 1905 ± 77 and 322 ± 27 sec^{-1} for intraburst, 61.8 ± 6.6 and 23.9 ± 5.8 sec^{-1} for interburst, 12.4 ± 6.0 and 13.6 ± 2.9 sec^{-1} for intercluster events, respectively. Intraburst kinetic properties of Kir6.2/SUR1 clones were essentially indistinguishable from pancreatic or cardiac K_{ATP} channel phenotypes, indicating that intraburst kinetics *per se* were insufficient to classify recombinant Kir6.2/SUR1 amongst native K_{ATP} channels. Yet, burst kinetic behavior of Kir6.2/SUR1 although similar to pancreatic, was different from that of cardiac K_{ATP} channels. Thus, expression of Kir6.2/SUR1 proteins away from the pancreatic microenvironment, confers the burst kinetic identity of pancreatic, but not cardiac K_{ATP} channels. This study reports the kinetic properties of Kir6.2/SUR1 clones which could serve in the further characterization of novel K_{ATP} channel clones.

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Key words: K_{ATP} channels — Kinetic model — Inward rectifier K^+ channel — ABC binding cassette — Pancreas — Cardiac

Introduction

Members of the ATP-sensitive K^+ (K_{ATP}) channel family are essential in coupling the metabolic state of a cell with membrane excitability in various tissues, yet their structure is partially understood (Aschcroft & Aschcroft, 1990; Nichols & Lederer, 1991; Takano & Noma, 1993; Findlay, 1994; Lazdunski, 1994; Terzic, Jahangir & Kurachi, 1995). Complementary DNAs (cDNAs) that encode putative subunits of K_{ATP} channels have been recently identified, and include clones for the sulfonylurea receptor (SUR), which belong to the ATP-binding cassette family of proteins, and clones for the K^+ inward rectifying channel, which belong to the Kir6.0 class of K^+ channels (Aguilar-Bryan et al., 1995; Inagaki et al., 1995, 1996; Nichols et al., 1996; Ämmälä et al., 1996*b;* Isomoto et al., 1996).

It was first shown that co-expression of SUR1 with Kir6.2 reconstitutes K_{ATP} channel-like activity with submillimolar sensitivity towards sulfonylureas, ATP and potassium channel opening drugs (Inagaki et al., 1995; Sakura et al., 1995). Neither Kir6.2 nor SUR1 alone could produce functional currents when heterologously expressed (Inagaki et al., 1995, 1996). Co-expression of certain Kir members with a SUR clone could also reproduce K^+ channel current with pharmacological properties related to native K_{ATP} channels (Ämmälä et al., 1996*a,b;* Gribble et al., 1997; Yamada et al., 1997). However, it is still not known whether putative channel subunits can operate within a specific set of conformational transitions that define the behavior of a particular K_{ATP} channel phenotype. Therefore, it is important to establish, in addition to general channel features, behavioral criteria useful to compare reconstituted with native K_{ATP} channels.

Such criteria could rely on single-channel kinetics which reflect properties of constitutive channel proteins. Several tissue-specific K_{ATP} channel phenotypes have previously been recognized due to different single-channel behavior (Trube and Hescheler, 1984; Zilberter et al., 1988; Gillis et al., 1989; Woll, Lönnendonker & Neumcke, 1989; Nichols, Lederer & Cannel, 1991; Takano & Noma, 1993). In this regard, co-expressed Kir6.2/SUR1 clones share identical intraburst behavior with pancreatic K_{ATP} channels (Inagaki et al., 1996). However, beyond intraburst behavior K_{ATP} channel activity possesses multiple conformational channel transitions which have not been defined for any of the co-expressed channel subunits nor related to a specific native K_{ATP} channel behavior.

Herein, we determine the complex single channel kinetics of heterologously expressed Kir6.2/SUR1 clones, and present a comparative kinetic analysis of recombinant with native pancreatic and cardiac K_{ATP} channel phenotypes. Data presented define kinetic properties of Kir6.2/SUR1 channel subunits, and support the notion that expression of these clones can confer specific pancreatic K_{ATP} channel behavior.

Materials and Methods

Transfection of Kir6.2 and SUR1 cDNA

Monkey kidney COS-7 cells were cultured (at 5% CO_2) in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, and seeded at 2×10^6 cells *per* 100-mm dish 24 hr prior to transfection (Kennedy, Nemec & Clapham, 1996). Full-length cDNAs, encoding mouse Kir6.2 (kindly provided by Dr. S. Seino, Chiba University) and hamster SUR1 (kindly provided by Drs. L. Aguilar-Bryan and J. Bryan, Baylor University), were subcloned into mammalian expression vectors pCMV6 and pCDNA1/amp, respectively (designated pCMV6-Kir6.2 and pCDNA1/amp-SUR). COS-7 cells were transiently transfected with plasmids using lipofectamine (GIBCO) according to the manufacturer's protocol. In brief, for each (100 mm) dish, pCMV6-Kir6.2 (4 μg) and pCDNA1/amp-SUR (4 μg) were included together with the expression plasmid vector for green fluorescent protein (2 μg of pGREEN-lantern; GIBCO) which served as a reporter gene for transfection (Marshall et al., 1995). Two microliters of lipofectamine reagent/μg of DNA provided the best transfection efficiency (~50%) and cell viability. The DNA/lipid mixture was incubated with cells for 5 hr in serum-free media. Approximately, 12 hr later COS-7 cells were lifted from the plate using PBS supplemented with 5 mM EDTA and replated onto glass coverslips at a 1:5 dilution for electrophysiological analysis which were performed at least 48 hr later.

Pancreatic Cells and Ventricular Cardiomyocytes

RIN-m5F insulin-secreting pancreatic cells were cultured (at 5% CO_2) in RPMI-1640 supplemented with 10% fetal calf serum, and cells plated onto glass coverslips 1–2 days prior to electrophysiological experiments. Ventricular myocytes were freshly isolated by enzymatic dissociation from guinea-pig hearts as described (Alekseev et al., 1996*a;* Elvir-Mairena et al., 1996).

Electrophysiological Measurements and Kinetic Analysis

Fire-polished pipettes, coated with Sylgard (resistance 8–10 MΩ), were filled with ''pipette solution'' (in mM): KCl 140, $CaCl_2$ 1, $MgCl_2$ 1, HEPES-KOH 5 (pH 7.3). Transfected COS cells, selected by green fluorescence under the microscope, as well as pancreatic RIN or cardiac cells were superfused with ''internal solution'' (in mM): KCl 140, $MgCl_2$ 1, EGTA 5, HEPES-KOH 5 (pH 7.3), and recordings made at room temperature (20–22°C) as described (Terzic et al., 1994*a,c;* Navarro et al., 1996; Terzic & Kurachi, 1996). Single-channel recordings were monitored on-line on a high-gain digital storage oscilloscope (VC-6025; Hitachi, Tokyo, Japan) and stored on tape using a PCM converter system (VR-10, Instrutech; New York, NY). Data were reproduced, low-pass filtered at 1 KHz (−3 dB) by a Bessel filter (Frequency Devices 902; Haverhill, MA), sampled at 80 μsec rate, and further analyzed using the ''BioQuest'' software (Alekseev et al., 1997; Brady et al., 1996).

For analysis of intraburst channel behavior, periods of channel ''silence'' that exceeded 3 msec were omitted. Using this criteria, close time distributions were well fitted by single exponents. For burst analysis, a burst in channel activity was defined as a set of opening and closures terminated by a close event with a duration that exceeded the critical time (t_{cutoff}), estimated based upon (Clapham & Neher, 1984; Gillis et al., 1989):

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

where τ_1, is the time constant of closed intervals within a burst; τ_2, the time constant of closed intervals between bursts; a_1 and a_2, areas of exponential fits corresponding to τ_1 and τ_2, respectively; t_d, ''dead time,'' i.e., time of underestimated events which equals the double sampling rate (~160 μsec). Based on such relationship, t_{cutoff} was estimated at ~2.5 msec, a value confirmed by detecting burst events in original current records. Fitting of closed and open time distributions by the sum of exponents (from 1 to 3) was carried out using minimization of the χ^2 criterion with the Nelder-Meed method of deformed polyhedron (Alekseev et al., 1996*b*). Results are expressed as mean ± SE; *n* refers to the number of experiments used in each analysis.

Results

Recombinant Kir6.2/SUR1, Pancreatic and Cardiac K_{ATP} Channel Phenotypes

Original channel records, obtained under analogous experimental conditions, in membrane patches excised from COS cells cotransfected with Kir6.2/SUR1 clones (upper trace), from pancreatic RIN cells (middle trace), and from ventricular cardiomyocytes (lower trace) are

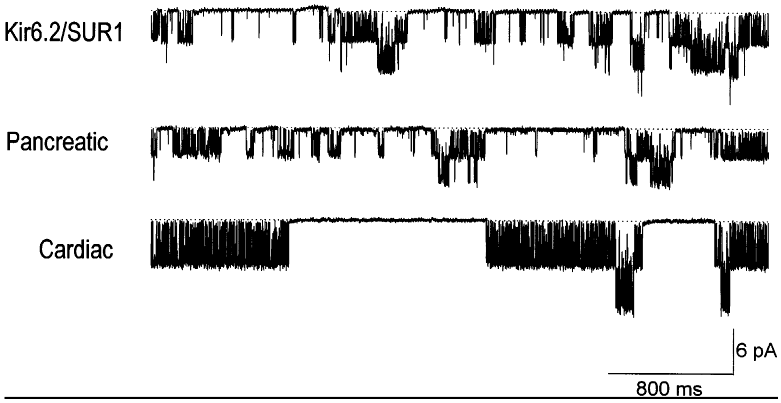

Fig. 1. Single-current records obtained in inside-out patches excised from: COS cell transfected with Kir6.2/SUR1 cDNAs (48 hr; upper trace), pancreatic RIN cell (middle trace), and ventricular cardiac cell (lower trace). Holding membrane potential: −60 mV. Dashed lines indicate zero-current level.

presented in Fig. 1. Amplitude histograms, constructed for given traces, revealed that single-channel amplitudes equaled 3.6, 3.4 and 5.4 pA in patches excised from COS-transfected, pancreatic, and cardiac cells, respectively. In agreement with previous studies, channel activities were inhibited by sulfonylurea drugs and ATP (*not illustrated*) as expected for K_{ATP} channel activity (Inagaki et al., 1995, 1996; Sakura et al., 1995; Tokuyama et al., 1996).

The K_{ATP} channel phenotype reconstituted by coexpressing Kir6.2/SUR1 clones into COS cells had a reversal potential virtually at 0 mV (under symmetrical, 140 mM, K^+ on the external and internal sides of patches), and a characteristic weak inward-rectifying feature (Fig. 2*A*). Linear regression of the current-voltage relationship at negative potentials revealed a single-channel conductance for the heterologously expressed Kir6.2/SUR1 channel activity of 58.4 ± 2.7 pS (n = 7; Fig. 2*A*). Analysis of voltage-current relationships of K_{ATP} channel currents obtained from pancreatic and cardiac cell-patches produced single-channel conductance of 57.2 ± 2.2 pS (n = 11) and 70–90 pS (Nichols & Lederer, 1991; Takano & Noma, 1993; Findlay, 1994; Terzic et al., 1995), respectively. In addition to exhibiting essentially identical single channel conductance, heterologously expressed Kir6.2/SUR1 clones and native pancreatic K_{ATP} channels apparently displayed similar kinetic behavior (Fig. 1).

Identical Intraburst Kinetics for Cloned and Native K_{ATP} Channel Activity

Single-channel records of co-expressed Kir6.2/SUR1 clones indicated that the apparent open-time duration of channel opening increased, whereas the frequency of channel closure decreased, as the membrane potential was progressively clamped from −100 to −20 mV (Fig. 2*B*). At these membrane potentials, open time histograms constructed for the Kir6.2/SUR1 heterologously expressed channel activity were fitted by single exponents, with τ_O equal to the mean open time. The mean open time of the recombinant Kir6.2/SUR1 channel activity displayed a voltage-dependence (Fig. 2*C*) characteristic of native K_{ATP} channels (Zilberter et al., 1988). Specifically, the mean open time for channel activity measured in patches from cells co-transfected with Kir6.2/SUR1 clones increased within the range of 1.9 ± 0.3 to 4.5 ± 0.8 msec (n = 5) between membrane potentials from −100 to −20 mV (Fig. 2*C*). Within the same range of membrane potentials, this compared closely to mean open time values obtained in patches from pancreatic RIN cells that increased from 2.01 to 8.5 msec (*not illustrated*), as well as to that from cardiac patches which increased from ~1.5 to 4.9 msec (*see also* Zilberter et al., 1988).

As the membrane potential was clamped from −100 to −20 mV, the distribution of intraburst closed-time of the Kir6.2/SUR1 channel activity could also be fitted by a single exponent. The voltage-dependence of the mean closed time was opposite to that of the mean open time, since mean closed time decreased from 0.7 ± 0.1 to 0.4 ± 0.03 msec between −100 and −20 mV. A similar voltage-dependence of the mean closed time was also observed for channel activity measured in pancreatic (from 0.75 to 0.41 msec; *not illustrated*) and cardiac cell-patches (from 0.8 to 0.4 msec; *see also* Zilberter et al., 1988).

At positive membrane potentials, open time distribution became a two-exponential phenomenon. When the membrane potential was progressively clamped from +20 to +80 mV the mean open time duration decreased from 12.1 ± 2.4 to 5.0 ± 0.2 msec for the Kir6.2/SUR1 clones expressed in COS cells (n = 5; Fig. 2*B* and *C*). Similarly, from +20 to +80 mV, the mean open time duration increased from 6.1 to 3.1 for K_{ATP} channels present in RIN cells (*not illustrated*), and from ≈11 to 2.8 msec for cardiac K_{ATP} channels (*see* Zilberter et al., 1988).

From +20 to +80 mV, the frequency of channel closure increased as the potential became more positive

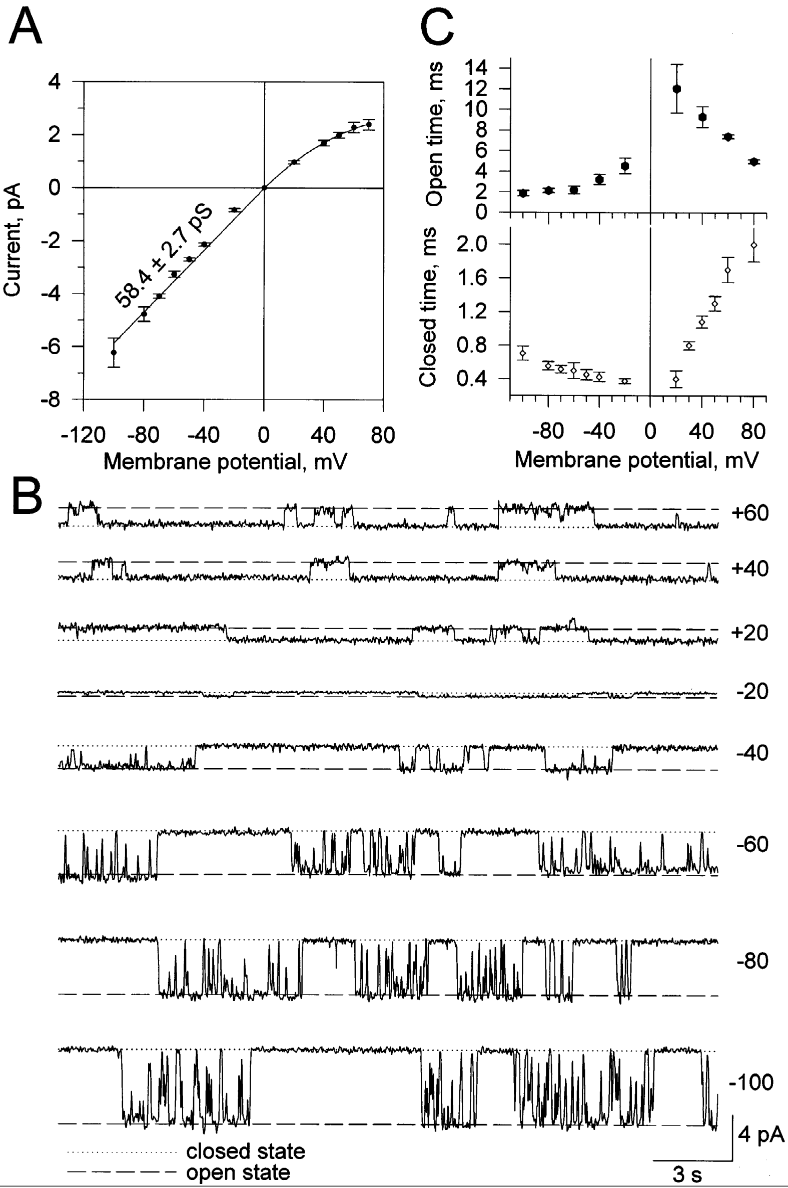

Fig. 2. Characteristics of Kir6.2/SUR1-expressed channel activity. (*A*) voltage-current relations of single-channel amplitude. (*B*) portions of representative single-channel records obtained at different membrane potentials (in mV; given on the right). Kinetic analysis was constructed from extended records. (*C*) voltage-current dependence for the mean open and closed times obtained from intraburst kinetic analysis. Vertical bars correspond to SE.

(Fig. 2*B* and *C*). The mean close time grew from 0.4 ± 0.1 to 2.01 ± 0.2 ms ($n = 5$) for the Kir6.2/SUR channel activity (Fig. 2*C*). Virtually identical voltage-dependency was also obtained for pancreatic RIN (from 0.51 to 1.1 msec; *not illustrated*) and cardiac K_{ATP} channels (from 0.2 to 0.5 msec; *see* Zilberter et al., 1988).

Burst Kinetics in Cloned vs. Native K_{ATP} Channels

Inspection of original channel traces indicated that channel openings occurred in bursts (Fig. 1). To further characterize channel behavior we, therefore, took into consideration the conventional assumption that transitions between the open (O) and the first closed (C_1) states represent transition within a burst (boxed in Scheme 1), whereas transitions between the open and the second closed (C_2) state define interburst kinetics[1]:

$$\boxed{C_1 \rightleftarrows O} \rightleftarrows C_2 \rightleftarrows \ldots \qquad (1)$$

Based upon defining a burst as a group of openings that

[1] Kinetics for pancreatic and cardiac K_{ATP} channels are usually represented by the C-O-C and the O-C-C schemes, respectively (Gillis et al., 1989; Kakei & Noma, 1984), yet both models appear adequate (Sakmann & Trube, 1984).

Table 1. Comparison of burst parameters between heterologously expressed Kir6.2/SUR1, RIN-pancreatic and cardiac K_{ATP} channel activity

	COS-Kir6.2/SUR1 (n = 5)	RIN-K_{ATP} (n = 3)	Cardiac-K_{ATP} (n = 7)
Mean burst duration, msec	17.9 ± 1.8	36.1 ± 4.6	212.5 ± 23.6
Mean closing per burst	5.6 ± 1.5	8.8 ± 1.1	98.7 ± 5.5

lasted at least 2.5 msec (t_{cutoff}; *see* Materials and Methods), the mean burst duration (i.e., burst length), and the mean number of closing *per* burst were calculated for each channel phenotype (Table 1). Obtained parameters indicated essentially two patterns—shorter burst duration with low number of closings *per* burst characteristic for recombinant Kir6.2/SUR1 and pancreatic K_{ATP} channel activity *vs.* (an order of magnitude) longer bursts with a higher number of closing events characteristic for the cardiac phenotype (Table 1; *see* also Fig. 1).

Kinetics of Kir6.2/SUR1 Channel Activity: Correlation with Pancreatic K_{ATP} Channels

Since COS cell-expressed Kir6.2/SUR1 and pancreatic K_{ATP} channel activity shared a common pattern of burst behavior, we further compared their kinetics. Heterologously co-expressed Kir6.2/SUR1 channel activity could not be described based upon two closed states, because the best fit of the closed time probability density function was obtained by applying a sum of three, rather than two, exponents (Fig. 3*A*). Therefore, in addition to intra- and interburst transitions, it was necessary to introduce the intercluster transition, related to the third closed state (C_3). This led to the following four-state linear kinetic scheme:

$$C_1 \underset{k_{21}}{\overset{k_{12}}{\rightleftarrows}} O \underset{k_{32}}{\overset{k_{23}}{\rightleftarrows}} C_2 \underset{k_{43}}{\overset{k_{34}}{\rightleftarrows}} C_3 \quad (2)$$

Within the frame of this model of channel behavior, application of burst analysis (t_{cutoff} = 2.5 msec) to recombinant Kir6.2/SUR1 channel activity (at −60 mV) revealed a single exponential distribution for gaps as well as openings within a burst (Fig. 4*A*). Characteristic times obtained from the distribution of open times and gaps within a burst were 2.8 ± 0.3 msec and 0.5 ± 0.03 msec (n = 5), respectively (Fig. 4*A*). These parameters, which reflect intraburst transition, were identical to values for open and close time distribution (2.2 ± 0.4 msec and 0.5 ± 0.1 msec, respectively at −60 mV) calculated, in the same patches, using intraburst kinetic analysis

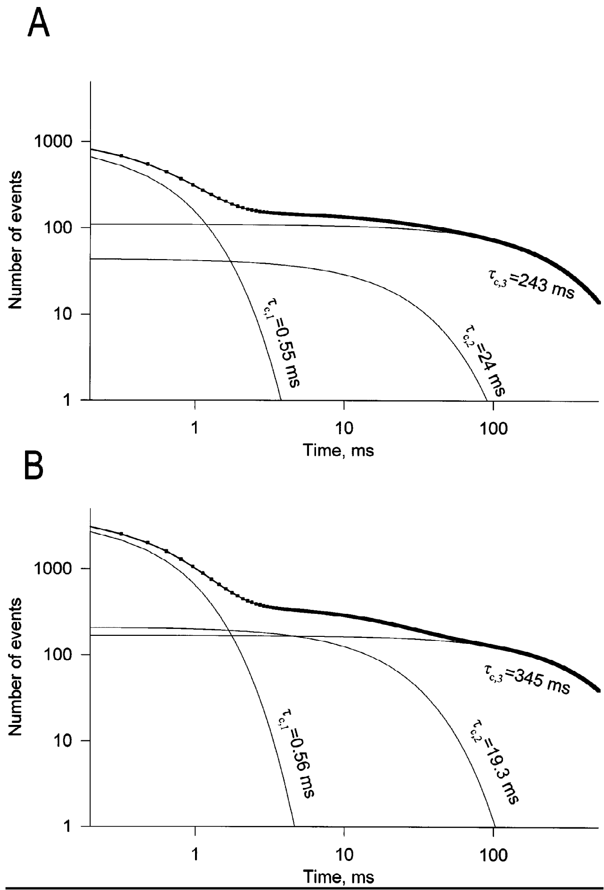

Fig. 3. Closed time distribution fitted by the sum of three exponents:

$$\psi_c\left(t\right) = \sum_{i=1}^{3} a_i \, exp\left(\frac{-t}{\tau_{c,i}}\right),$$

where a_i is the relative area under each exponent normalized to 1 (i.e., Σa_i = 1), and $\tau_{c,i}$, respective time constants (*A*) For Kir6.2/SUR1-reconstituted channel activity in transfected COS cell; (*B*) For pancreatic-RIN K_{ATP} channel activity. Both distributions are presented using a log-log scale. Results of fitting are plotted as solid lines through data points. Curves labeled with characteristic time values representing the three exponent components.

(Fig. 2*C; see* Materials and Methods). This indicates that the t_{cutoff} value used to identify a burst was adequate.

The four-state linear kinetic model (scheme 2) used for heterologously expressed Kir6.2/SUR channel activity was also appropriate to describe native pancreatic K_{ATP} channel behavior (*see also* Gillis et al., 1989). As in the case of reconstituted channel activity (Fig. 3*A*), and using identical burst criteria, the closed time probability density function for K_{ATP} channel activity recorded from pancreatic RIN-cells was also fitted by the sum of three exponents (Fig. 3*B*), whereas the probability density function for gaps and opening within a burst had also one-exponential (t_{cutoff} = 2.5 msec; Fig. 4*B*).

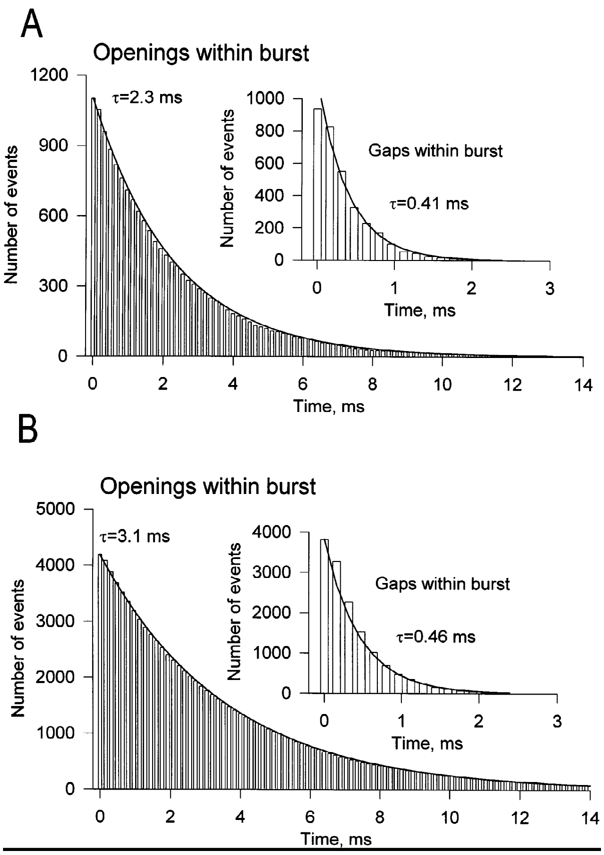

Fig. 4. Open time (and gap, *inset*) distribution within a burst obtained based on burst analysis and fitted by single exponents:

$$\psi_o\left(t\right) = exp\left(\frac{-t}{\tau_o}\right)$$

where τ is the respective time value. (*A*) For Kir6.2/SUR1-reconstituted channel activity in transfected COS cell; (*B*) For pancreatic-RIN K_{ATP} channel activity. Results of fitting are plotted as solid lines through data points, and labeled with specific time constants.

Parameters, which define open and closed time distribution, i.e., three close characteristics times ($\tau_{c,1}$, $\tau_{c,2}$, $\tau_{c,3}$) with representative relative area under each exponents (a_1, a_2, a_3), and a characteristic open time (τ_0), were similar for recombinant Kir6.2/SUR1 and pancreatic K_{ATP} channels (Table 2).

Based upon the common model (scheme 2), and using the set of parameters that define open and closed time distribution (*see* Table 2), rate constants corresponding to intraburst, interburst and intracluster transitions were calculated using the following relationships (*see also* Gillis et al., 1989):

$$k_{12} = 1/\tau_{c,1}$$
$$k_{21} = a_1/\tau_0$$
$$k_{23} = A/\tau_0$$

Table 2. Parameters defining open and closed time distribution of channel activity recorded in representative patches from a COS cell co-expressed with Kir6.2/SUR1 clones and from a pancreatic RIN cell

	Cos-Kir6.2/SUR1	RIN-K_{ATP}
Gaps within a burst ($\tau_{c,1}$), msec	0.55	0.56
Gaps between bursts ($\tau_{c,2}$), msec	24.0	19.3
Gaps between clusters ($\tau_{c,3}$), msec	243	345
Relative area of $\tau_{c,1}$ (a_1)	0.86	0.91
Relative area of $\tau_{c,2}$ (a_2)	0.04	0.05
Relative area of $\tau_{c,3}$ (a_3)	0.10	0.04
Open time (τ_o), msec	2.3	3.1

$$k_{32} = a_2/(A \cdot \tau_{c,2})$$
$$k_{34} = a_3/(A \cdot \tau_{c,2})$$
$$k_{43} = A/(a_2 \cdot \tau_{c,3} - A \cdot \tau_{c,2})$$

where $A = a_1 + a_2$. On average, we found that not only k_{21} and k_{12} rates, which reflect intraburst transitions, but also k_{23} and k_{32} rates, which represent interburst activity, as well as values for k_{34} and k_{43} rates, which relate to intercluster interactions, were similar for heterologously co-expressed Kir6.2/SUR1 clones and RIN-cell pancreatic K_{ATP} channels (Table 3). Moreover, these values compared closely with previously reported rates defining K_{ATP} channels in other types of pancreatic β-cells (Gillis et al., 1989; *see* Table 3).

Discussion

The present study determines the kinetic parameters defining the behavior of recombinant Kir6.2/SUR1 channel activity, and provides an additional criterion to identify Kir6.2/SUR1 clones within the K^+ channel superfamily. Such parameters may serve in the further characterization of novel K_{ATP} channel clones.

Native K_{ATP} channels display characteristic voltage-dependence of the mean open and closed times, a feature common to other inward rectifying K^+ channels (Sakmann & Trube, 1984; Kurachi, 1985; Terzic, Jahangir & Kurachi, 1994*b*). Therefore, the first aim was to test whether the Kir6.2/SUR1 channel activity also possesses voltage-dependent kinetics expected for an inward rectifying K^+ channel. The present study did reveal that the Kir6.2/SUR1 channel activity displayed such voltage-dependence which manifested as a symmetrically opposed change in the mean open and closed times at negative *vs.* positive membrane potentials (*see* Fig. 2*C*), and was comparable to that obtained for RIN-pancreatic (*present study*) and cardiac K_{ATP} channels (Zilberter et al., 1988). Thus, in addition to inward-rectification, recombinant Kir6.2/SUR1 channel activity shared the voltage-dependent channel kinetics characteristic of the K^+ inward rectifying family (Sakmann & Trube, 1984).

Table 3. Rate constants for the four-state kinetic model calculated from channel activity recorded in patches from COS cells co-expressed with Kir6.2/SUR1 clones and from a RIN-insulinoma cell (present study), and compared to previously reported values from pancreatic β cells

	COS-Kir6.2/DUR1 (n = 5)	RIN-K_{ATP}	β cell-K_{ATP} (Gillis et al., 1989)
k_{12}	1904.6 ± 77.3	1785.7	2197.8
k_{21}	322.2 ± 27.4	293.5	559.9
k_{23}	61.8 ± 6.6	29.0	28.3
k_{32}	23.9 ± 5.8	28.8	30.0
k_{34}	12.4 ± 6.0	23.0	3.60
k_{43}	13.6 ± 2.9	5.8	3.2

Furthermore, intraburst kinetics of Kir6.2/SUR1 clones were similar to native K_{ATP} channels not only in terms of voltage-dependence, but also in terms of indistinguishable mean open and closed time values recorded at various holding membrane potentials (Gillis et al., 1989; Zilberter et al., 1988; Takano & Noma, 1993; Terzic et al., 1995). Thus, equivalent intraburst behaviors were obtained for channels expressed in COS cells *vs.* pancreatic and/or cardiac cells (present study; Inagaki et al., 1996). This indicates that Kir6.2/SUR1 clones share intraburst channel properties which appear conserved within the K_{ATP} channel family, and independent from the tissue environment in which channels are expressed.

Inspection of long-lasting channel behavior revealed significant differences in the burst duration among investigated K_{ATP} channel phenotypes (Fig. 1). Specifically, the burst duration and the number of closings *per* burst that characterize Kir6.2/SUR1 channel activity were close to the pancreatic, but an order of magnitude reduced when compared to cardiac K_{ATP} channels (Table 1). Thus, to distinguish specific K_{ATP} channel phenotypes analysis of channel activity needs to be extended from intraburst to burst kinetic description.

As Kir6.2/SUR1 showed close similarity with pancreatic K_{ATP} channels, further kinetic analysis was performed within the framework of the four-state linear model (Scheme 2) previously established to describe the burst kinetic behavior of pancreatic channels (Gillis et al., 1989). This four-state model was adequate for the analysis of recombinant channel activity as closed-time distributions of Kir6.2/SUR1 activity were well fitted by a sum of three exponents (Fig. 3 and Table 2). Calculated rates for transitions between intraburst, interburst, and intercluster states defining the overall Kir6.2/SUR1 channel behavior were similar to those obtained for RIN (*present study*) and pancreatic β-cell (Gillis et al., 1989) K_{ATP} channels. Thus, burst behavior of co-expressed Kir6.2/SUR1 activity corresponds to native K_{ATP} channels found in various pancreatic preparations. In turn, this could indicate that, in contrast to intraburst kinetics, parameters defining burst behavior could be used as a kinetic ''fingerprint'' for a particular K_{ATP} channel phenotype.

The significance of measuring rates of interburst and intercluster transitions lies not only in defining a specific kinetic behavior, but also in the precise understanding of the mechanisms of regulation of K_{ATP} channel activity. Indeed, it has been shown that inhibitory ligands that gate K_{ATP} channel opening, such as sulfonylurea drugs and ATP, affect the rates of interburst and intercluster transitions (Gillis et al., 1989; Nichols et al., 1991; Takano & Noma, 1993; Terzic, Tung & Kurachi, 1994*d*). Data presented herein can, thus, provide baseline values for the further characterization of ligand-dependent gating of the Kir6.2/SUR1 kinetics.

Thus, in addition to previously described elementary biophysical properties, nucleotide and pharmacological regulation, as well as coupling to the cellular metabolic state (Inagaki et al., 1995; Nichols et al., 1996; Tokuyama et al., 1996; Gribble et al., 1997), the present study defines the complex burst kinetics of recombinant Kir6.2/SUR1 channel activity. Comparison of this kinetic behavior with native K_{ATP} channel phenotypes revealed that: (1) Kir6.2/SUR1, pancreatic, and cardiac channel phenotypes were indistinguishable in terms of intraburst kinetic behavior; (2) Kir6.2/SUR1 channel activity was similar to pancreatic, but different from the cardiac channel phenotype in terms of burst duration and/or number of closing *per* burst; (3) Kir6.2/SUR1 and pancreatic channel activities displayed identical overall burst behavior. The present data, thus, indicate that pancreatic-like K_{ATP} channel kinetic phenotype could be reconstituted outside the pancreas by heterologous co-expression of Kir6.2/SUR1 cDNAs. Indistinguishable intraburst kinetics within the investigated K_{ATP} channel phenotypes are suggestive of uniform properties of the pore-forming proteins constitutive to these channels, yet interburst differences may relate to nonuniform properties of the regulatory channel subunit, such as analogues of the SUR1 protein (Inagaki et al., 1996; Isomoto et al., 1996).

This work was sponsored through the ''Bruce and Ruth Rappaport Program in Vascular Biology and Gene Delivery.'' A.T. is a recipient of research grants from the Miami Heart Research Institute, the American Heart Association, the George M. Einseberg Cardiovascular Research Fund, and the Harrington Professorship Fund. M.E.K. and B.N. are supported, in part, through fellowships from NRSA and COLCIENCIAS.

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