Analysis of Single \(K_{\text{ATP}}\) Channels in Mammalian Dentate Gyrus Granule Cells

MARC R. PELLETIER,1,2 PETER A. PAHAPILL,3 PETER S. PENNEFAATHER,5 AND PETER L. CARLEN1,2,4

1Blooview Epilepsy Research Laboratory, Division of Cellular and Molecular Biology, Toronto Western Research Institute; and Departments of 1Physiology, Medicine (2Neurosurgery, 3Neurology), and 5Pharmaceutical Science, University of Toronto, University Health Network, Toronto, Ontario MST 2S8, Canada

Received 16 February 2000; accepted in final form 28 July 2000

INTRODUCTION

ATP-sensitive \(K^+\) (\(K_{\text{ATP}}\)) channels are sensors of cellular metabolic activity. Information concerning the metabolic state is transduced into electrical information, which can influence the excitability of the cell membrane: an increase in the ATP/ADP ratio closes \(K_{\text{ATP}}\) channels, which produces membrane depolarization (for reviews, see Ashcroft and Ashcroft 1990; Babenko et al. 1998; Bryan and Aguilar-Bryan 1997; Davies et al. 1991; Quayle et al. 1997; Seino 1999). The distribution of \(K_{\text{ATP}}\) channels is widespread, and in peripheral tissues, they are present on cardiac myocytes (Noma 1983), pancreatic \(\beta\) cells (Cook and Hales 1984), vascular smooth muscle (Zhang and Bolton 1996), and skeletal muscle (Spruce et al. 1985). Their distribution in the brain is also widespread, having been identified in striatal cholinergic interneurons (Lee et al. 1998), midbrain dopaminergic neurons (Guatteo et al. 1998), CA1 pyramidal neurons, interneurons of the stratum radiatum, glia, and dentate gyrus granule cells (Zawar et al. 1999), globus pallidus and ventral pallidum (Gehlert et al. 1991), substantia nigra (Mourre et al. 1991), and pituitary (Bernardi et al. 1993).

Activation of \(K_{\text{ATP}}\) channels has been linked to a variety of physiological functions including maintenance of resting membrane potential and inhibition of insulin secretion in pancreatic \(\beta\) cells (Cook et al. 1988), inhibition of dopamine release (Zhu et al. 1999), volatile anesthetic-induced coronary arteriole dilation (Zhou et al. 1998), both termination and re-initiation of seizure activity (Klöcker et al. 1996) and postischemia cardioprotection (Bernardo et al. 1999) and neuroprotection (Lauritzen et al. 1997; Reshef et al. 1998; Takaba et al. 1997; Wind et al. 1997), and resistance against the chronic metabolic stress-induced neurodegeneration observed in Parkinson’s and Huntington’s diseases (Beal 1996; Hanna and Bhatia 1997).

Several studies have demonstrated that \(K_{\text{ATP}}\) channel activation is protective against experimental ischemia/reperfusion-induced damage (Guatteo et al. 1998; Heurteaux et al. 1993; Wind et al. 1997); this supports further the hypothesis that activation of \(K_{\text{ATP}}\) channels exists as a potentially important therapeutic intervention for myocardial and cerebral ischemia (Cason et al. 1995; Grover 1997). The CA1 area of the hippocampus is a region of the brain that is particularly vulnerable to ischemia/reperfusion-induced damage (Smith et al. 1984).
Interestingly, the dentate gyrus (DG), which is the site of the first synapse of the hippocampal trisynaptic circuit and thus can exert a powerful influence on the transfer of information in the hippocampus, possesses a higher density of K\textsubscript{ATP} channels (Karschin et al. 1997) and is an area in the brain that is relatively resistant to ischemia/reperfusion-induced damage (Krnjević and Ben-Ari 1989). Therefore we were interested in describing the characteristics of single K\textsubscript{ATP} channels in DG granule cells. In this study, we have characterized the pharmacology and the kinetics of single K\textsubscript{ATP} channels recorded in cell-attached patches from DG granule cells in acutely prepared rat brain slices.

METHODS

Slice preparation and electrophysiology

Male Wistar rats (15–25 days of age) were anesthetized with halothane (Halocarbon Laboratories, River Edge, NJ) and then decapitated. The brain was removed rapidly and placed for approximately 1 min in ice-cold, oxygenated (95% O\textsubscript{2}-5% CO\textsubscript{2}) artificial cerebrospinal fluid (ACSF) containing (in mM) 126 NaCl, 3.5 KCl, 2 CaCl\textsubscript{2}, 2 MgSO\textsubscript{4}, 26 NaHCO\textsubscript{3}, 1.25 NaH\textsubscript{2}PO\textsubscript{4}, and 10 glucose. A block of brain containing the hippocampus was fixed to an aluminum chuck using cyanoacrylate glue, and then coronal hippocampal slices (400 μm) were prepared with a Vibratome. After an incubation period of at least 1 h, slices were transferred to an interface-type chamber and perfused (1–2 ml/min) with oxygenated ACSF containing (in mM) 120 NaCl, 5 KCl, 2 CaCl\textsubscript{2}, 2 MgSO\textsubscript{4}, 26 NaHCO\textsubscript{3}, 10 glucose, 10 TEA, 2 CsCl, 1 4-aminopyridine (4-AP), and 0.0005 TTX. Experiments were conducted at room temperature (20–22°C).

Single K\textsubscript{ATP} channel activity was recorded using an Axopatch 200B amplifier in voltage-clamp mode (patch configuration) in cell-attached patches from DG granule cells with thick-walled (0.66 mm) borosilicate glass pipettes (World Precision Instruments; Sarasota, FL) that were pulled on a vertical electrode puller (Narishige, PP-83). Pipettes had resistances of 6–8 MΩ when filled with a solution containing (in mM) 140 KMeSO\textsubscript{4}, 2 CaCl\textsubscript{2}, 0.0002 charybdotoxin (CTX), 10 TEA, 2 CsCl, 1 4-AP, and 0.01% BSA (300 mOsm, pH 7.3 with KOH). In accordance with the instructions supplied by the manufacturer (Alomone Labs, Jerusalem, Israel), CTX was prepared first as a stock solution (1 μM), which contained (in mM) 100 NaCl, 10 Tris, 1 EDTA, and 0.1% BSA. Pipettes were positioned and lowered manually into the middle of the granule cell layer using a hydraulic micromanipulator (Narishige; WR-60). The electrode holder port was connected via tubing to a three-way stopcock, a 3-ml syringe, and a sphygmonanometer. Constant positive pressure (10 mmHg) was maintained during the incremental descent into the DG cell body layer. When a cell was approached, application, followed by the release, of negative pressure (40–60 mmHg) routinely produced high-resistance seals between the pipette and the cell membrane ranging from 6 to 10 GΩ.

It has been well documented that there is a reduction over time, referred to as rundown, of single K\textsubscript{ATP} channel activity when electrophysiological recordings are made from excised patches, a phenomenon that has been generally attributable to the disruption, or loss, of intracellular regulatory elements (Quayle et al. 1997). Therefore we recorded single K\textsubscript{ATP} channel activity in the cell-attached configuration, which preserves the integrity of the intracellular milieu. In the cell-attached configuration, a positive pipette potential (V\textsubscript{p}) causes the transmembrane potential (V\textsubscript{m}) to become more negative or hyperpolarized. Our pipette solution contained 140 mM KMeSO\textsubscript{4}, allowing an intracellular concentration of 140 mM K\textsuperscript{+}, the reversal potential for K\textsuperscript{+} (E\textsubscript{K}) predicted by the Nernst equation will be 0 mV. The single-channel openings we recorded reversed polarity when the V\textsubscript{p} was equal to approximately −70 mV, which served as an estimation of the resting membrane potential (RMP). To test this directly, we performed whole cell voltage-clamp recordings of DG granule cells (n = 6) with pipettes containing (in mM) 140 KMeSO\textsubscript{4}, 10 HEPES, 2 MgATP, and 0.1 EGTA (270 mOsm, pH 7.3 with KOH). By measuring the zero-current membrane potential and the current response produced by a hyperpolarizing voltage step (−10 mV, 200-ms duration), we obtained values of −64.8 ± 1.7 (SE) mV and 112.2 ± 3.6 mV for the RMP and the input resistance, respectively.

Data were acquired at a sampling frequency of 5 kHz, recorded on video tape (VR-10; Instrotech), filtered off-line at 2 kHz with an eight-pole Bessel filter (Frequency Devices, Haverhill, MA), then digitized (Axotape 2.02). Records were first idealized, then analyzed, using the Fetchan and the pSTAT programs, respectively, of pCLAMP version 6.0.3 software (Axon Instruments, Foster City, CA). All records were inspected visually followed by either manual or automatic event detection using the 50% current amplitude as the threshold. Analyses were performed on continuous records of single-channel activity ranging in duration from 20 to 120 s. Amplitude histograms were fitted with Gaussian distributions using the Simplex least-squares method. Bursts of single-channel openings were defined as groups of channel openings separated by closed periods five times longer than the time constant for the brief closed times (Rowe et al. 1996). This value served as the step test value and the burst delimiter in the analysis of interburst interval and burst duration, respectively. Burst duration was also assessed manually from both the digitized and the idealized records, which produced similar results. The optimal interburst interval was determined by plotting the number of channel closings per burst versus time (Sigurdson et al. 1987). Open and closed dwell-time histograms were plotted with logarithmic time axes, which were scaled to minimize vacant bins, then fitted using the maximum-likelihood-estimation method (Sigworth and Sine 1987). Inspection of the residuals revealed that they were typically distributed symmetrically above and below zero and were of small value. To promote the quality of fit, fitting limits were selected that did not include those bins with large residual values. The significance of kinetic models of increasing order was determined by assessing the logarithm of the likelihood ratio. The estimation of kinetic parameters was based on approximately 9,800 (control, 11 patches), 5,400 (diazoxide, 5 patches), and 7,640 (hypoxia/hypoglycemia, 6 patches) single-channel events. Statistical comparisons were made with the Student’s t-test. Differences were considered significant if P < 0.05. Data are presented as means ± SE.

Drugs

Glybenclamide (Sigma-Aldrich Canada, Oakville, Ontario), tolbutamide (Sigma-Aldrich Canada), and diazoxide (Tocris Cookson, Ballwin, MI) were dissolved in dimethyl sulfoxide, stored frozen as aliquots of a stock solution [in mM] 10, 100, and 1000, respectively, then diluted in ACSF to the experimental concentration and bath applied. These drugs reach their targets by partitioning into the lipid phase of the membrane (Kozlowski et al. 1989) and have been demonstrated previously to be effective in cell-attached patches (Sturgess et al. 1988; Trube et al. 1986).

Hypoxia/hypoglycemia

To induce metabolic stress, we used a conventional hypoxia-hypoglycemia protocol, which comprised exposing the slices for 5 min to ACSF that was bubbled with 95% N\textsubscript{2}-5% CO\textsubscript{2} and that had sucrose substituted for glucose (Duffy and MacVicar 1996; Perez Velazquez et al. 1997).

RESULTS

Immediately after switching to the patch configuration and V\textsubscript{p} was equal to 0 mV, we observed outwardly directed single-
channel openings. A representative example of the single-channel openings is presented in Fig. 1A. Amplitude histograms were fit best with the sum of two Gaussians and showed that single separate closed and open states could be distinguished clearly for this channel, which is illustrated in Fig. 1B. The amplitude of the single-channel outward current decreased progressively as \( V_p \) became more negative until no channel openings were observed when \( V_p \) was between \(-60 \) and \(-70 \) mV. When \( V_p \) was more negative than \(-70 \) mV, single-channel openings were reversed in polarity; this is consistent with values for RMP and \( E_K \) equal to approximately \(-70 \) and \( 0 \) mV, respectively. Patches typically contained a single active channel, and we recorded similar single-channel activity in 59/105 patches. Representative records of single-channel openings in a patch at magnitudes of \( V_m \), ranging from \(-90 \) to \(+10 \) mV are presented in Fig. 1C.

We did not observe any indication of current rundown during the experiments, and in one patch, single-channel activity was recorded for 80 min, the longest time recorded. Linear regression analysis of the current/voltage relation produced a unitary slope conductance of 27 pS, which is presented in Fig. 1D. The probability of channel opening (\( P_{\text{open}} \)) when \( V_m \) was equal to \(-70 \) mV was \(0.04 \pm 0.01\), which was not different when compared with the \( P_{\text{open}} \) when \( V_m \) was equal to \(+10 \) mV (0.038 \( \pm 0.01\); 5 patches), suggesting that channel activation was voltage-independent.

Single-channel openings were organized into well-delineated bursts, which were apparent from visual inspection of both the digitized and the idealized records. To characterize the burst behavior, we determined first the interburst interval, which represents the minimum value of a closed duration that separates the bursts. The optimal interburst interval was \(286.8 \pm 13.1 \) ms, which is consistent with a frequency of \(3.5 \pm 0.8 \) Hz. Burst duration ranged from 8.6 to 48.7 ms, with a mean duration of \(19.3 \pm 1.6 \) ms. At a greater gain and faster time scale, as is presented in Fig. 1B (inset), it can be seen that the open channel returns briefly to the closed state within a burst, which is consistent with two types of channel closure: brief closures within a burst and long closures that determine the interburst interval. Similar burst kinetics for single \( \text{K}_{\text{ATP}} \) channels have been published previously (Ashcroft et al. 1988; Kakei and Noma 1984; Rorsman and Trube 1985; Spruce et al. 1985).

**Single-channel openings were blocked by sulfonylureas**

One pharmacological criterion used in the identification of \( \text{K}_{\text{ATP}} \) channels is inhibition by sulfonylureas (Babenko et al. 1998). Tolbutamide and glybenclamide are first- and second-generation sulfonylureas, respectively. Glybenclamide is more efficacious compared with tolbutamide for producing hypoglycemia and is used clinically in the treatment of hyperglycemia associated with Type II diabetes mellitus. Sulfonylureas produce hypoglycemia via a direct blockade of the sulfonylurea receptor (SUR) associated with \( \text{K}_{\text{ATP}} \) channels located on pancreatic \( \beta \)-cells, which results in an increase in the release of insulin (Panten et al. 1989; Schmid-Antomarchi et al. 1987). Glybenclamide and tolbutamide have been demonstrated to block \( \text{K}_{\text{ATP}} \) channels in a variety of tissues (Gopalakrishnan et al. 1999; Katnick and Adams 1997; Sturgess et al. 1985; Zhang and Bolton 1996; Zünkler et al. 1988) and, as stated in the preceding text, are considered to be prototypic blockers of \( \text{K}_{\text{ATP}} \) channels. Therefore we were interested in assessing whether glybenclamide and tolbutamide would block the putative single \( \text{K}_{\text{ATP}} \) channels we had recorded.

Single-channel activity was blocked in 2/6 patches by the...
channel activity after bath application of diazoxide (100 μM). In 5/5 patches, we observed an increase in single-muscle K<sub>ATP</sub> channels (Ashcroft and Ashcroft 1990; Seino 1999). The activation of single-channel activity by diazoxide was rapid and occurred with a latency ranging from 2 to 3 min. A representative example of the single-channel activation produced by diazoxide is presented in Fig. 3. Diazoxide increased significantly <i>P<sub>open</sub></i> to 0.12 ± 0.02. The duration of bursts in the presence of diazoxide was 15.7 ± 3.1 ms, which was not different when compared with control; however, we did observe a twofold increase in the frequency of bursts to 8.3 ± 2.0 Hz, which was significantly greater when compared with control. Additionally, the increased burst behavior appeared to be organized in clusters with a duration of several seconds and with an intercluster interval of 20–30 s. There were no differences attributable to diazoxide for either the <i>E<sub>K</sub></i> or the conductance of the single-channel openings (3/3 patches).

### Single-channel activity was increased by hypoxia/hypoglycemia

Several studies have demonstrated that K<sub>ATP</sub> channels are activated by hypoxia, which produces an initial hyperpolarization of the <i>V<sub>m</sub></i>, often by more than 10 mV (Fujimura et al. 1997; Krnjević and Leblond 1989) and is thought to contribute to the blockade of synaptic transmission associated with hypoxia (Fujiwara et al. 1987; Hansen et al. 1982; Mourre et al. 1989; Yamamoto et al. 1997). We used a conventional protocol to induce hypoxia/hypoglycemia in brain slices. Specifically, slices were exposed for 5 min to ACSF that was bubbled with 95% N<sub>2</sub>-5% CO<sub>2</sub> and that had sucrose substituted for glucose. In 6/7 patches, we observed an increase in single-channel activity after 2–3 min of exposure to hypoxia/hypoglycemia. Similar to the effect produced by diazoxide, hypoxia/hypoglycemia produced increases in both <i>P<sub>open</sub></i> (0.09 ± 0.03) and burst frequency (7.1 ± 1.7 Hz), which were significantly different when compared with control. There was no difference in burst duration (19.5 ± 3.9 ms) when compared with control. The
hypoxia/hypoglycemia-induced activation of single $K_{\text{ATP}}$ channels persisted for up to 30 min after reperfusion with control ACSF, the longest time we recorded. A representative example of the hypoxia/hypoglycemia-induced activation of single $K_{\text{ATP}}$ channels is presented in Fig. 4.

There were no differences attributable to the hypoxia/hypoglycemia challenge in either the $E_K$ or the conductance of the single channels (4/4 patches). Application of glybenclamide (10 $\mu$M) during the reperfusion was without effect (3/3 patches) on the hypoxia/hypoglycemia-induced activation of $K_{\text{ATP}}$ channels (data not shown). Although the activation of $K_{\text{ATP}}$ channels by various methods is often blocked successfully by sulfonylureas (e.g., Fujimura et al. 1997; Guatteo et al. 1998; Lee et al. 1998), our observation of a loss of effectiveness of sulfonylureas on metabolic stress-induced $K_{\text{ATP}}$ channel activity has been reported previously in ventricular myocytes (Findlay 1993). Because of the rapid onset (2–3 min), modification of the protocol that we used to induce hypoxia-hypoglycemia might permit the demonstration of blockade by sulfonylureas of the metabolic stress-induced increase in channel activity, e.g., repeated 1-min exposures to hypoxia-hypoglycemia alternating with 5-min episodes of return to control ACSF.

**Single-channel kinetics of $K_{\text{ATP}}$ channels in DG granule cells**

Cell-attached patches typically contained only a single active channel, which permitted the estimation of parameters for a kinetic model. The open-state histograms could be fitted with...
a single exponential, and the fit was not improved significantly using the sum of two exponentials. The closed-state histograms in contrast required the sum of two exponentials to obtain an adequate fit, which was not improved significantly, by the sum of three. Representative examples of open and closed dwell-time histograms for the three recording conditions are presented in Fig. 5. The kinetic analyses suggest that the single K\textsubscript{ATP} channel activity we recorded in DG granule cells can be characterized by a kinetic model with at least a single fast open state with a time constant ($\tau_{\text{open}}$) of $1.3 \pm 0.2$ ms, a fast closed state with a time constant ($\tau_{\text{closed, fast}}$) of $2.6 \pm 0.9$ ms, and a second, much longer, closed state with a time constant ($\tau_{\text{closed, slow}}$) of $302.7 \pm 66.7$ ms. A summary of the channel kinetics for the single K\textsubscript{ATP} channels we recorded is presented in Table 1. Activation of K\textsubscript{ATP} channels by either diazoxide or hypoxia/hypoglycemia had no effect on either $\tau_{\text{open}}$ or $\tau_{\text{closed, fast}}$ but decreased significantly $\tau_{\text{closed, slow}}$ by an order of magnitude, which is consistent with the significant increase in burst frequency we observed for these treatments.

**DISCUSSION**

We have recorded single-channel activity in DG granule cells from acutely prepared rat brain slices with pharmacological, electrophysiological, and kinetic features consistent with what has been published previously to be characteristic of K\textsubscript{ATP} channels. The single-channel activity we recorded demonstrated the following: an $E_{\text{K}}$ close to 0 mV, consistent with the $E_{\text{K}}$ predicted by the Nernst equation for symmetrical K\textsuperscript+ solutions; a low $P_{\text{open}}$, which was voltage-independent; open-

![FIG. 5. Histograms of open (left) and closed (right) dwell-time distributions of single K\textsubscript{ATP} channel activity recorded from cell-attached patches (V\textsubscript{m} = -70 mV) and exposed to either control conditions (A), diazoxide (B), or hypoxia/hypoglycemia (C). For all groups, a single exponential was required to fit open dwell-time histograms and the sum of 2 exponentials was required to fit closed dwell-time histograms. Note, that the $\tau_{\text{closed, slow}}$ for the examples representing single-channel activity after exposure to diazoxide and hypoxia/hypoglycemia are an order of magnitude more brief when compared with control, which represents shorter inter-burst intervals, and hence, an increase in the frequency of bursts. Numbers in parentheses represent number of openings or closings, where appropriate, that comprise the distribution.

| Table 1. Summary of kinetics of single K\textsubscript{ATP} channels recorded in cell-attached patches from DG granule cells |
|---|---|---|---|---|---|
| | n | $P_{\text{open}}$ | Burst Duration, ms | Burst Frequency, Hz | $\tau_{\text{open}}$, ms | $\tau_{\text{closed, fast}}$, ms | $\tau_{\text{closed, slow}}$, ms |
| Control | 9800 | 0.04 ± 0.01 | 19.3 ± 1.6 | 3.5 ± 0.8 | 1.3 ± 0.2 | 2.6 ± 0.9 | 302.7 ± 66.7 |
| Diazoxide | 5400 | 0.12 ± 0.02* | 15.7 ± 3.1 | 8.3 ± 2.0* | 1.0 ± 0.1 | 1.4 ± 0.3 | 24.4 ± 2.6* |
| Hypoxia/Hypoglycemia | 7640 | 0.09 ± 0.03* | 19.5 ± 3.9 | 7.1 ± 1.7* | 0.9 ± 0.1 | 1.6 ± 0.4 | 29.5 ± 1.8* |

Values are means ± SE. n represents the number of single-channel events used in the estimation of the kinetic parameters. * $P < 0.05$, unpaired t-test.
ings that were organized into bursts; a unitary conductance of 27 pS; blockade by the sulfonylureas, glybenclamide, and tolbutamide; and activation by both diazoxide and by hypoxia/hypoglycemia-induced metabolic stress.

Included in the two points is fulfillment of two pharmacological criteria that are generally accepted as being indicative of K$_{ATP}$ channels (Babenko et al. 1998). As stated in the preceding text, sulfonylureas represent a class of hypoglycemics that increase insulin release by direct inhibition of K$_{ATP}$ channels on pancreatic $\beta$ cells. Sensitivity to sulfonylureas is determined by the SUR subunit and differences reflect channels composed of SUR1 (high affinity) and SUR2A.2B (low affinity) subunits. The relative potency of sulfonylureas has been studied in detail in SUR1-based channels in $\beta$ cells where glybenclamide ($K_i = 4$ nM) is three orders of magnitude more potent when compared with tolbutamide ($K_i = 4$ $\mu$M) in inhibiting whole cell currents (Zünkl et al. 1988). The $K_i$ for glybenclamide and tolbutamide in pancreatic $\beta$ cells has generally been reported to be in the low nanomolar and the low micromolar range, which is two orders of magnitude lower when compared with the $K_i$ for SUR2A-based channels in cardiac muscle (Davies et al. 1991). We selected high concentrations of sulfonylureas to maximally block K$_{ATP}$ channel activity and did not investigate systematically a concentration-response relation for the blockade of single-channel activity produced by glybenclamide and tolbutamide. The concentrations of glybenclamide (10 $\mu$M) and tolbutamide (20, 100 $\mu$M) we used are similar to those used in previous studies attempting to characterize K$_{ATP}$ channels (e.g., Fujimura et al. 1997; Gopalakrishnan et al. 1999; Guatteo et al. 1998; Jiang and Haddad 1997; Katnick and Adams 1997; Lee et al. 1998). Consistent with previous observations (Gillis et al. 1989; Liss et al. 1999; Zünkl et al. 1989), the effect produced by glybenclamide in our experiments was slower and irreversibly compared with tolbutamide, which was faster and reversible.

In contrast to what we observed, fast block kinetics with subconductance states, i.e., resolvable conductance levels that are intermediate between $I_{closed}$ and $I_{open}$ channels, have not been reported previously for glybenclamide. Unlike tolbutamide, Hill coefficients greater than unity, e.g., 1.8 (Zünkl et al. 1988) and 1.5 (Sturgess et al. 1986), have been published previously for glybenclamide, which is consistent with ligand-receptor interactions more complex than mass action, e.g., cooperativity. The transient subconductance state we observed might be attributable to ligand/receptor interactions between glybenclamide molecules and a novel splice variant of the SUR subunit comprising native DG granule cell K$_{ATP}$ channels, e.g., transition to a conformational state that partially occludes the pore region. Subconductance kinetics has been described for a variety of ligand-gated ion channels and might represent a fundamental mode of ion channel operation (Gage 1988). Blocking reactions where subconductance behavior has been observed in other channels include the blockade of Na$^+$, large-conductance Ca$^{2+}$-dependent $K^+$ (Maxi K$^+$) and skeletal muscle mechanosensitive channels by veratridine (Wang et al. 1990), dendrotoxin (Lucchesi and Moczydlowski 1990), and the aminoglycoside antibiotic, neomycin (Winegar et al. 1996), respectively.

The single-channel conductance of K$_{ATP}$ channels (symmetrical high K$^+$) reported previously are wide ranging and can be divided into small/medium (20–100 pS)- and large-conductance (more than 100 pS) channels. Based on this classification, the K$_{ATP}$ channels, we recorded in DG granule cells reside at the lower end of the conductance range for small/medium-conductance channels. The majority of the K$_{ATP}$ channels described thus far have been small/medium-conductance channels and have been recorded in vascular smooth muscle including rabbit portal vein (25 pS) (Kamouchi and Kitamura 1994) and pig coronary artery (35 pS) (Dart and Standen 1995), cardiac muscle (80 pS) (Findlay 1988; Noma 1983), and pancreatic $\beta$ cells (50–65 pS) (Ashcroft and Kakei 1989; Cook and Hales 1984; Misler et al. 1986). Large-conductance channels have been recorded in ventromedial hypothalamic neurons (150 pS) (Ashford et al. 1990) and rabbit renal arterioles (258 pS) (Lorenz et al. 1992).

The single K$_{ATP}$ channels we recorded had a low open-state probability ($P_{open}$ of 0.04 ± 0.01), which was voltage independent. These observations are consistent with what has been generally reported for K$_{ATP}$ channels (Dart and Standen 1995; Davies et al. 1991; Qin et al. 1989; Rorsman and Trube 1985; Zhang and Bolton 1995). Voltage-dependent channel activity, which increases with membrane depolarization, has also been reported (Hunter and Giebisch 1988; Spruce et al. 1985; Sturgess et al. 1987). The activity of K$_{ATP}$ channels has been generally described as being relatively insensitive to Ca$^{2+}$ (Ashcroft and Ashcroft 1990); however, a type of K$_{ATP}$ channel associated with epithelial cells is activated by micromolar concentrations of Ca$^{2+}$ (Hunter and Giebisch 1988). We did not assess the Ca$^{2+}$-dependence of the K$_{ATP}$ channels we recorded.

In comparison to the members of the inwardly rectifying $K^+$ channel (Kir) 2.0, 3.0, and 4.0 subfamilies (Douplnik et al. 1995), which show strong inward rectification, members of the Kir6.0 subfamily confer only weak inward rectification, which requires depolarization to potentials greater than +50 mV (Inagaki et al. 1995b). Similar to other Kir-based channels, the rectification of K$_{ATP}$ channels is mediated by a voltage- and a $K^+$-gradient-dependent block by internal ions such as Na$^+$ and Mg$^{2+}$ (Horie et al. 1987; Woll et al. 1989). We did not observe inward rectification of the single-channel openings we recorded. The most depolarized potential at which we recorded single-channel activity was +30 mV, which may have been insufficient to produce much rectification. Additionally, recording conditions with symmetrical high concentrations of K$^+$ typically produce a near linear unitary current/voltage relation (Quayle et al. 1997).

**Subunit composition of native K$_{ATP}$ channels**

K$_{ATP}$ channels are heteromultimer complexes of an ion channel and a receptor that are structurally unrelated (for reviews, see Babenko et al. 1998; Bryan and Aguilar-Bryan 1997; Seino 1999). Subunits comprising functional K$_{ATP}$ channels are members of the Kir and the ATP-binding cassette protein (SUR) superfamilies, which are associated as tetramers with a 1:1 stoichiometry. The Kir tetramer forms the pore region and determines the channel conductance (Kir6.1-based channels possess smaller unitary conductance), whereas, the SUR tetramer determines the sensitivity to nucleotides and to sulfonylurea drugs (Inagaki et al. 1996). The recent cloning of Kir6.0 and of SUR has led to the identification of Kir6.1, Kir6.2 (Inagaki et al. 1995a,b; Sakura et al. 1995) and SUR1.
(high affinity), SUR2 (Aguilar-Bryan et al. 1995; Chutkow et al. 1996; Inagaki et al. 1996; Isomoto et al. 1996) subunits, respectively. Additionally, two splice variants of SUR2 (also referred to as SUR2A) have been identified and are referred to as SUR2B and SUR2C (Ashcroft and Gribble 1998).

The subunit composition of \( K_{ATP} \) channels native to DG granule cells is not known. The electrophysiological (small conductance), pharmacological (blockade by sulfonylureas, activation by diazoxide), and physiological (activation by metabolic stress) characteristics of the single-channel activity we recorded are consistent with what has been described previously for Kir6.1/SUR1-, SUR2B-based channels. Because both SUR1- and SUR2B-based channels are activated by diazoxide, the use of selective K\(^+\) channel openers (KCOs) might differentiate between these two candidates. The KCOs, pinacidil, cromakilim, and nicorandil, are far more selective for SUR2B-based channels, having little, or no, effect in SUR1-based channels in pancreatic \( \beta \) cells. On the other hand, both SUR1- and SUR2B-based channels have been observed in the same cell type, e.g., dopaminergic substantia nigra neurons (Liss et al. 1999). To unequivocally identify the subunit composition of native channels requires a combination of pharmacological and molecular techniques. The \( K_{ATP} \) channels we recorded appear to be distinct from those channels found in both rat and in human neocortical neurons where two distinct types have been described: S-\( K_{ATP} \), small conductance (47 pS), strong inward rectification (symmetrical K\(^+\)), glybenclamide sensitive and L-\( K_{ATP} \), large conductance (200 pS), weak rectification, glybenclamide sensitive, and Ca\(^{2+}\) (\( \mu M \)) dependent (Jiang and Haddad 1997).

It is known that different cell types display differential susceptibility to metabolic stress such as that produced by hypoxia, that activation of \( K_{ATP} \) channels is protective against ischemia/reperfusion-induced damage and that individual cells may express different \( K_{ATP} \) channels (Zawar et al. 1999). Therefore it is important to elucidate the subunit composition of the critical \( K_{ATP} \) channels in the resistant cells. By using a combination of whole cell recording, pharmacology, and RT-multiplex PCR, Liss et al. (1999) reported that \( K_{ATP} \) channels in dopaminergic midbrain neurons express both SUR1 and SUR2B subunits in combination with a Kir6.2 subunit and importantly, the Kir6.2/SUR1 combination confers the greatest protection against metabolic stress.

**Single-channel kinetics of \( K_{ATP} \) channels**

A prominent feature of \( K_{ATP} \) channel activity is that the channel openings are organized into bursts (Ashcroft et al. 1988; Davies et al. 1992; Inagaki et al. 1996; Kamouchi and Kitamura 1994; Rorsman and Trube 1985; Spruce et al. 1987). Burst kinetics characteristic of single \( K_{ATP} \) channels can be described in simple terms as bursts of openings separated by longer interburst intervals (Ashcroft and Ashcroft 1990). Differences in burst kinetics have been attributed to the \( K_{ATP} \) subunits: SUR2A-based channels display longer burst duration and interburst intervals when compared with SUR1-based \( \beta \)-cell \( K_{ATP} \) channels (Inagaki et al. 1996).

Indicative of burst behavior, the closed dwell-time histograms constructed for the single \( K_{ATP} \) channels we recorded were fitted best with the sum of two exponentials and consisted of a fast closed state, with a time constant (\( \tau_{closed,fast} \)) of 2.6 ± 0.9 ms, which represents the duration of the channel closings within a burst, and a second, much longer, closed state with a time constant (\( \tau_{closed,slow} \)) of 302.7 ± 66.7 ms, which represents the duration of the much longer interburst interval. Our analyses suggest that single \( K_{ATP} \) channel activity in DG granule cells is consistent with a kinetic model with at least a single fast open state, with a \( \tau_{open} \) of 1.3 ± 0.2 ms, and at least two closed states. The kinetic behavior of \( K_{ATP} \) channels is complex and not understood fully. A variety of kinetic models have been proposed previously including models with a single open and a single closed state (Qin et al. 1989), at least two closed states (Ashcroft et al. 1988), and at least two open and three closed states (Davies et al. 1992; Spruce et al. 1987). A fourth, long-duration closed state has also been proposed (Davies et al. 1991), which is reminiscent of the increased burst behavior produced by diazoxide that we observed where the increase in frequency of bursts appeared to be nested together in clusters separated by long-duration intercluster intervals. The long-duration closed states are difficult to resolve due primarily to their low frequency and requires long-duration recording of channel activity.

Application of ATP to the intracellular face of an excised patch containing \( K_{ATP} \) channels results in a reduction of channel activity, which in cardiac myocytes has been attributed to a prolongation of the interburst interval and to a shortening of the burst duration (Kakei and Noma 1984). A similar mechanism was described for the reduction of single \( K_{ATP} \) channel activity produced by glucose in pancreatic \( \beta \) cells (Ashcroft et al. 1988). Munemori et al. (1996) reported that in inside-out patches of frog ventricular myocytes, the decrease in \( P_{open} \) produced by ATP was attributable solely to an increase the interburst interval. The activation of single \( K_{ATP} \) channels in DG granule cells produced by diazoxide and hypoxia/hypoglycemia we observed was attributable not to an increase in burst duration but to a decrease in \( \tau_{closed,slow} \), which represents the interburst interval and is consistent with an increase in the frequency of bursts.

**Physiological roles of \( K_{ATP} \) channels**

The tissue distribution of \( K_{ATP} \) channels is widespread, and their physiological roles are equally diverse. The coupling of cellular metabolism to membrane excitability produces a variety of functional consequences in different tissues, e.g., regulation of local blood flow via effects on smooth muscle tone and appetite control via effects on ventromedial hypothalamus neurons (Ashcroft and Ashcroft 1990). In addition to being regulated by the intracellular concentration of ATP, \( K_{ATP} \) channels are modulated by a variety of mechanisms including phosphorylation (Light 1996) and intracellular pH (Davies et al. 1992). This is particularly relevant in cardiac and skeletal muscle when even during vigorous activity the concentration of intracellular ATP is maintained at levels (5–10 mM) that are below the IC\(_{50}\) for ATP, which is attributable to the buffering action of creatine phosphate and creatine kinase (Carlson and Siger 1960).

In light of their ubiquitous expression, ability to couple metabolism to membrane excitability, and potential for modulation by a variety of variables, it is not surprising that the therapeutic potential of targeting \( K_{ATP} \) channels has been investigated in a variety of clinical conditions, e.g., reperfusion-
induced cell death and chronic metabolic stress-induced neurodegeneration implicated in Parkinson’s and Huntington’s diseases (Lawson 2000). K_{ATP} channels localized in pancreatic β cells have been studied most and exist presently as an important therapeutic target in the management of Type II diabetes. Glucose blocks the activity of these K_{ATP} channels in a concentration-dependent manner and results in membrane depolarization and the activation of voltage-dependent Ca^{2+} channels (VDCCs), which produces a pattern of electrical activity consisting of slow oscillations in membrane potential between depolarized plateau phases with superimposed action potentials (bursts) and repolarization phases (Ashcroft and Rorsman 1989). While the voltage- and the Ca^{2+}-dependent inactivation kinetics of VDCCs contribute to the interburst interval, it appears that the direct modulation of K_{ATP} channel activity is the primary mechanism for determining both the duration and the frequency of bursts. Additionally, pancreatic β cells within an islet of Langerhan are electrically coupled via gap junctions (Meda et al. 1986). Gap junctional communication permits the transmission of electrical information rapidly throughout a local syncytium of cells, which helps to synchronize the activity of the coupled cells. Initiation of the electrical activity is attributable to pacemaker cells, which possess characteristics that promote intrinsic bursting, e.g., depolarized V_{m}, lower spike threshold or higher glucose affinity.

**K_{ATP} channel activity and seizures**

The electrical behavior of β cells is reminiscent of the paroxysmal depolarizing shifts (PDSs) recorded intracellularly from principal cells in disinhibited brain slice preparations. The PDS is a common feature of in vitro seizure models, and the clinical correlate, the interictal discharge, is an electroencephalographic feature of an epileptic brain (Hughes 1989; Jensen and Yaari 1988; Matsumoto and Marsan 1964). Interestingly, the same mechanisms, i.e., intrinsic membrane properties that predispose cells to behave as pacemakers and electrical coupling via gap junctions, promote synchronous activity in neurons and are believed also to participate in various aspects of seizure activity (McNamara 1994; Perez Velazquez and Carlen 2000; Prince and Connors 1986); however, the role of K_{ATP} channels in seizure activity has not been investigated systematically. Because of the ubiquitous expression of K_{ATP} channels in the brain and their role in the maintenance of the RMP, in regions of the brain where there is a high density of K_{ATP} channels in neurons coupled by gap junction, modulation of these K_{ATP} channels might influence seizure activity. Activation of the channels would hyperpolarize the V_{m} thus taking the membrane further from the threshold for activation of VDCCs and firing of action potentials. The former would decrease Ca^{2+} influx, which might prevent the initiation of Ca^{2+}-dependent cell-death cascades (Pelletier et al. 1999). The latter might serve to increase the seizure threshold of these cells or to limit the propagation of the discharge. Another suggestive link between K_{ATP} channel and seizure activity is the influence of pH. Acidification of the intracellular pH has been demonstrated to reduce the duration of epileptiform activity (Xiong et al. 2000), an effect that is thought to be mediated in part by the closure of gap junctions (Perez Velazquez et al. 1994). In skeletal muscle K_{ATP} channels, intracellular acidification increased by an order of magnitude the K_{s} for ATP (Davies et al. 1992), an effect that would increase P_{open} which would hyperpolarize the V_{m} or produce a shunt current, or both, consistent with an anticonvulsant influence. Conversely, mechanisms that inhibit K_{ATP} channels might initiate an oscillation of the RMP, then following recruitment and synchronization of a sufficient population of excitable cells, a local epileptiform discharge would be initiated that may or may not be propagated further.

The ability to influence membrane excitability also raises the potential that modulation of K_{ATP} channels might also influence the voltage-dependent mechanisms underlying nerve conduction and the release of neurotransmitters (Farnas et al. 2000). Therefore it seems likely that K_{ATP} channels localized in various regions of the brain might exist as important therapeutic targets, and knowledge concerning their molecular composition would promote the development of drugs with selective actions on different tissues.

We thank F. Vidic for technical support.

This work was supported by grants from the Medical Research Council of Canada (P. A. Pahapill, P. S. Pennefather, and P. L. Carlen) and the Blooreview Epilepsy Program (M. R. Pelletier and P. L. Carlen).

**REFERENCES**


Doupnik CA, Davidson N, and Lester HA. The inward rectifier potassium channel.


