# Sulfonylurea Sensitivity of Adenosine Triphosphate–Sensitive Potassium Channels From β Cells and Extrapancreatic Tissues

Fiona M. Gribble and Frances M. Ashcroft

**Sulfonylureas are widely used to stimulate insulin secretion in type 2 diabetic patients because they close adenosine triphosphate–sensitive potassium ($K_{ATP}$) channels in the pancreatic β-cell membrane. This action is mediated by binding of the drug to the sulfonylurea receptor (SUR1) subunit of the channel. $K_{ATP}$ channels are also present in a range of extrapancreatic tissues, but many of these contain an alternative type of SUR subunit (SUR2A in heart and SUR2B in smooth muscle). The sulfonylurea-sensitivity of $K_{ATP}$ channels containing the different types of SUR is variable: gliclazide and tolbutamide block the β cell, but not the cardiac or smooth muscle types of $K_{ATP}$ channels with high affinity. Glibenclamide and glimepiride, on the other hand, block channels containing SUR1 and SUR2 with similar affinity. The reversibility of the different sulfonylureas also varies. Tolbutamide and gliclazide produce a reversible inhibition of Kir6.2/SUR1 and Kir6.2/SUR2 channels, whereas glibenclamide has a reversible effect on cardiac, but not β-cell, $K_{ATP}$ channels. In this article, we summarize current knowledge of how sulfonylureas act on $K_{ATP}$ channels containing the different types of sulfonylurea receptor, and discuss the implications of these findings for the use of sulfonylureas in the treatment of diabetes mellitus.**


ADENOSINE TRIPHOSPHATE–sensitive potassium ($K_{ATP}$) channels are the targets for the sulfonylurea drugs used in the treatment of type 2 diabetes mellitus.[1] They are found in a wide range of tissues, including pancreatic β cells, some central neurons, and cardiac, smooth, and skeletal muscle. A principal role for $K_{ATP}$ channels is to couple the metabolic state of a cell to its electrical excitability. In β cells, $K_{ATP}$ channels provide a link between the glucose concentration and the rate of insulin secretion. In cardiac muscle, the channels open during hypoxia and may play a role in reducing ischemic injury by shortening the cardiac action potential.[2] $K_{ATP}$ channels in vascular smooth muscle are involved in the regulation of vessel tone,[3] and, in skeletal muscle, $K_{ATP}$ channel opening is triggered by fatigue.[4] A distinct form of the $K_{ATP}$ channel is located in the inner membrane of mitochondria. This channel appears to play a role in the ischemic preconditioning of cardiac myocytes.[5]

## ROLE OF THE β-CELL $K_{ATP}$ CHANNEL

$K_{ATP}$ channels in the pancreatic β cell play an important role in the response to physiologic stimuli such as glucose, and to pharmacologic agents such as the sulfonylureas and $K_{ATP}$ channel openers.[6] In β cells, $K_{ATP}$ channels are open at low glucose concentrations, and the tendency for potassium ions ($K^+$) to leave the cells through open K channels results in a negative resting membrane potential of approximately −70 mV. Glucose blocks the channels as a consequence of its metabolism. A rise in the plasma glucose concentration increases the rate of glycolysis and results in enhanced ATP synthesis and a concomitant decrease in the adenosine diphosphate (ADP) concentration. Both these adenine nucleotides are believed to play a role in regulating $K_{ATP}$ channel activity: the principal effect of ATP is to inhibit the channels, whereas MgADP is a channel activator. The increase in ATP and fall in ADP therefore act together to close $K_{ATP}$ channels. As the potassium efflux ceases, the membrane potential begins to depolarize and triggers the opening of voltage-gated calcium channels. The resultant calcium entry stimulates the release of insulin-containing vesicles.

## $K_{ATP}$ CHANNELS IN OTHER EXCITABLE TISSUES

$K_{ATP}$ channels in other tissues such as nerve and muscle are also sensitive to adenine nucleotides. In these tissues, however, changes in nucleotide concentrations may reflect the availability of oxygen more than that of glucose. A fall in the ATP concentration in response to hypoxia results in $K_{ATP}$ channel opening, and the consequent $K^+$ efflux tends to hyperpolarize the plasma membrane. In vascular smooth muscle, this enhances muscle relaxation,[3] and in cardiac muscle it results in shortening of the cardiac action potential.[2]

## $K_{ATP}$ CHANNEL STRUCTURE AND DIVERSITY

$K_{ATP}$ channels comprise an octameric complex of pore-forming Kir6.x subunits and regulatory sulfonylurea receptors (SURs).[7-10] Four Kir6.2 subunits combine to form the channel pore, but the resultant tetramer remains trapped in the endoplasmic reticulum (ER) in the absence of SUR. Excision of an ER retention signal from Kir6.2 allows the channels to reach the surface membrane in vitro, and under these conditions it can be shown that Kir6.2 is directly inhibited by ATP in the absence of SUR.[11,12] Sulfonylurea receptors enable the normal trafficking of Kir6.2, and endow the channels with sensitivity to sulfonylureas and to activation by Mg-nucleotides and $K_{ATP}$ channel openers.[11] SUR is a member of the family of ATP-binding cassette (ABC) transporter proteins, a group that also includes the cystic fibrosis transmembrane conductance regulator (CFTR) and the multiple drug resistance proteins. Like other ABC transporters, SUR possesses multiple transmembrane (TM) domains, and two large intracellular nucleotide binding do-

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mains (NBDs), each with a Walker A and Walker B nucleotide binding motif.[7,13]

Two different genes encoding SURs (SUR1 and SUR2) have been identified, and further diversity is produced by alternative splicing of SUR2.[14,15] The β-cell $K_{ATP}$ channel is formed from Kir6.2 and SUR1 subunits. Cardiac and skeletal muscle channels, in contrast, contain Kir6.2 and SUR2A, and smooth muscle $K_{ATP}$ channels are formed by the coupling of SUR2B with either Kir6.2 or Kir6.1.[16]

## TISSUE SPECIFICITY OF SULFONYLUREAS

As a result of this molecular diversity, $K_{ATP}$ channels from different tissues exhibit a range of pharmacologic properties. Of particular relevance to diabetic clinical practice is the finding that the sensitivity to sulfonylurea inhibition depends on the SUR subtype. We have examined the effects of sulfonylureas on $K_{ATP}$ channels from different tissues by expressing cloned channels in *Xenopus* oocytes, and recording macroscopic currents in excised membrane patches (Fig 1 and Table 1).

Gliclazide blocks the β-cell type of $K_{ATP}$ channel with high affinity (50% inhibitory concentration [$IC_{50}$] ~50 nmol/L) by interaction with the SUR1 subunit. In experiments on membrane patches in the absence of added nucleotide, high-affinity gliclazide block of Kir6.2/SUR1 channels reduces the current amplitude by approximately 50%.[17] Complete current inhibition is only observed at suprapharmacologic concentrations of the

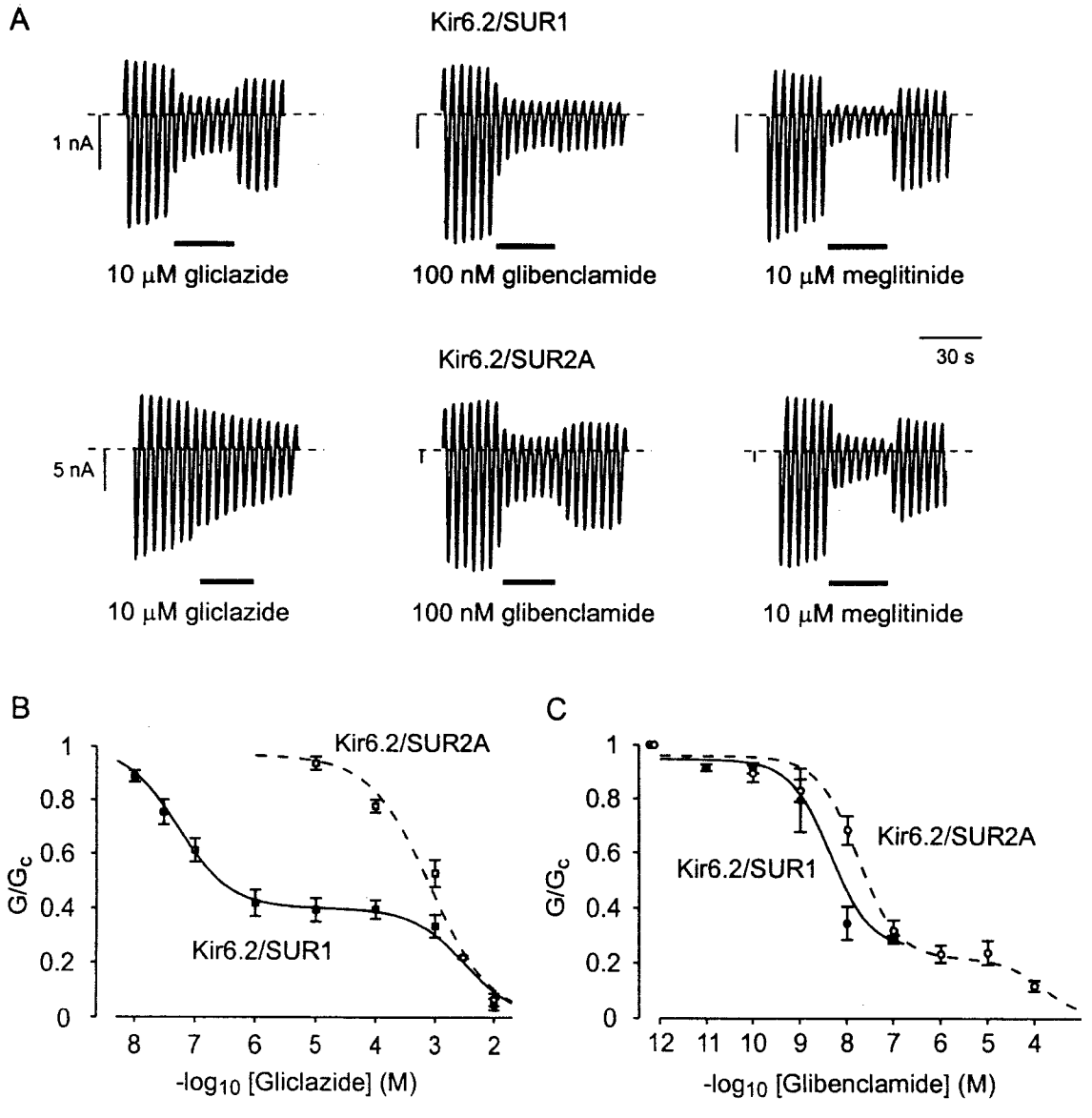


**Fig 1. Inhibition of β-cell and cardiac types of $K_{ATP}$ channel by gliclazide, glibenclamide, and meglitinide. (A) Effects of gliclazide (10 μmol/L), glibenclamide (100 nmol/L), or meglitinide (10 μmol/L) on cloned β-cell (Kir6.2/SUR1) and cardiac (Kir6.2/SUR2A) $K_{ATP}$ channels. (B, C) Concentration-response relationships for gliclazide (B) and glibenclamide (C) inhibition of Kir6.2/SUR1 and Kir6.2/SUR2A currents. The conductance in the presence of the sulfonylurea (G) is expressed as a fraction of that in the absence of the drug ($G_c$). Oocytes were coinjected with mRNAs encoding Kir6.2 and either SUR1 or SUR2A, and macroscopic currents recorded from inside-out patches in response to a series of voltage ramps from −110 mV to +100 mV. Data from Gribble et al.[17,19]**

**Table 1. Sulfonylurea Sensitivities of Cloned β-Cell and Cardiac $K_{ATP}$ Channels**

| | Kir6.2/SUR1 | | Kir6.2/SUR2A | |
|---|---|---|---|---|
| | High Affinity | Low Affinity | High Affinity | Low Affinity |
| Gliclazide | 50 nmol/L | 3 mmol/L | — | 0.8 mmol/L |
| Tolbutamide | 5 μmol/L | 2 mmol/L | — | 1.7 mmol/L |
| Glibenclamide | 4 nmol/L | ND | 27 nmol/L | 110 μmol/L |

NOTE. $IC_{50}$ values for high- and low-affinity inhibition of Kir6.2/SUR1 and Kir6.2/SUR2A currents from references 17 and 19.

Abbreviation: ND, not done.

drug. At these levels, gliclazide may act directly on the Kir6.2 subunit, as it is effective even when Kir6.2 is expressed in the absence of SUR.[17]

Glibenclamide and tolbutamide also block Kir6.2/SUR1 currents at high- and low-affinity sites, but differ from gliclazide in both affinity and reversibility.[18,19] Tolbutamide blocks Kir6.2/SUR1 currents with lower affinity than gliclazide (~5 μmol/L compared with ~50 nmol/L), and is readily reversible. Glibenclamide is more potent ($IC_{50}$ ~4 nmol/L), but its effect is largely irreversible in the time course of our experiments.

Unlike Kir6.2/SUR1 currents, Kir6.2/SUR2A currents are not blocked by gliclazide and tolbutamide with high affinity, and show only the low-affinity block mediated by Kir6.2. Therapeutic concentrations of gliclazide and tolbutamide are therefore unlikely to affect $K_{ATP}$ channels in cardiac, skeletal, and smooth muscle, which are formed from Kir6.2 and SUR2 subunits. Glibenclamide, on the other hand, inhibits both Kir6.2/SUR1 and Kir6.2/SUR2A currents with high affinity ($IC_{50}$s of ~4 nmol/L and ~27 nmol/L, respectively).[19] However, glibenclamide block of the cardiac type of $K_{ATP}$ channel differs from that of the β-cell type in being largely reversible. Like glibenclamide, glimepiride blocks both Kir6.2/SUR1 and Kir6.2/SUR2A currents with high affinity, and its effect on Kir6.2/SUR1 currents is largely irreversible (D.K. Song and F.M. Ashcroft, unpublished observations, November 1999).

## REVERSIBILITY OF SULFONYLUREA INHIBITION

The differences we observe in the reversibility of sulfonylurea block may be explained by the idea that molecules such as glibenclamide interact with SUR1 at 2 sites, whereas tolbutamide and gliclazide interact with a single site. Glibenclamide is made up of 2 parts: one half resembles tolbutamide and contains the sulfonylurea group; the other half possesses a benzamido group (Fig 2). Meglitinide is a drug that resembles the benzamido moiety of glibenclamide, and is itself able to block both Kir6.2/SUR1 and Kir6.2/SUR2A currents with high affinity (Fig 1).[19] We therefore propose that glibenclamide blocks Kir6.2/SUR2A currents by interaction at a single site (the benzamido site), but that it interacts with 2 sites (one benzamido and one sulfonylurea) on SUR1.[19] The simultaneous interaction of a glibenclamide molecule with 2 sites may decrease the likelihood of drug dissociation, and result in the irreversibility of the drug in the time-course of electrophysiologic experiments. The irreversibility of glibenclamide action observed in our experiments may also explain why, in [$^3$H]-glibenclamide–binding studies, the drug binds with high affinity to SUR1 but not apparently to SUR2A.[14]

## INTERACTIONS BETWEEN SULFONYLUREAS AND NUCLEOTIDES

Although the results described above suggest that tolbutamide and gliclazide may provide greater β-cell specificity than glibenclamide, the situation becomes more complicated when the effects of nucleotides are taken into consideration. It has been recognized for several years that sulfonylureas inhibit β-cell $K_{ATP}$ channels more effectively when nucleotides are included in the solution perfusing the intracellular membrane surface.[20] This may be explained by the finding that sulfonylureas have 2 separate actions on β-cell $K_{ATP}$ channels. One effect, that described above, is the direct inhibitory action of the drug observed in the absence of nucleotide. The second effect, however, is their ability to prevent the stimulatory action of Mg-nucleotides such as MgADP.[18] These 2 effects appear to be distinct, since truncation of the N-terminus of Kir6.2 can abolish the direct inhibitory action of tolbutamide without disrupting its interaction with MgADP.[21] The prevention of MgADP activation by sulfonylureas is not observed with Kir6.2/SUR2A currents. In fact, MgADP appears to reduce glibenclamide inhibition of the cardiac type of $K_{ATP}$ channel.[19,22]

The interaction between sulfonylureas and nucleotides is likely to play a major role in the action of the drugs in vivo, since $K_{ATP}$ channels are normally exposed to a mixture of cytoplasmic nucleotides. The enhanced effect of sulfonylureas in the presence of MgADP may explain why complete inhibition of Kir6.2/SUR1 channels is observed when the drugs are tested on intact cells, whereas they block currents in isolated membrane patches by only about 50%. In cardiac muscle, the presence of MgADP would have the opposite effect, tending to reduce inhibition by glibenclamide.[22] It is therefore possible that, under ischemic conditions, when MgADP levels are high and cardiac $K_{ATP}$ channels are open, glibenclamide may have very little effect on channel activity. However, it is also possible that other nucleotides, whose interactions with sulfonylureas have not been tested, may influence the response in intact cells.

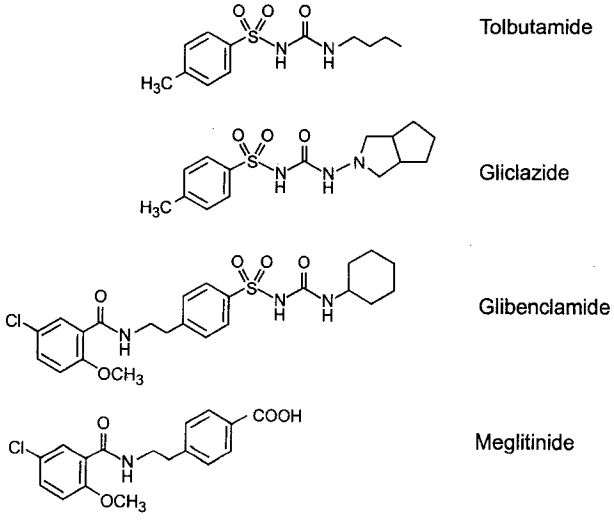


**Fig 2. Chemical structures of tolbutamide, gliclazide, glibenclamide, and meglitinide.**

## PHYSIOLOGIC CONSEQUENCES OF $K_{ATP}$ CHANNEL BLOCKADE

The ability of sulfonylureas to stimulate insulin secretion has been appreciated for many years. The effectiveness of sulfonylureas on β cells arises because the $K_{ATP}$ channel is partially open, and therefore potentially blockable, in the resting β cell.

The situation may be different in cardiac muscle, as recordings from intact cardiac myocytes show that $K_{ATP}$ channels in these cells are largely blocked under normal physiologic conditions.[2] Sulfonylureas might not, therefore, affect Kir6.2/SUR2A channels in cardiac muscle under normal working conditions when the channels are already blocked, but may become effective when the channels open during ischemia. A clearer understanding of the physiologic role of the cardiac $K_{ATP}$ channel is required before the potential side effects of sulfonylurea action on the heart can be fully predicted.

The clinical consequences of blocking extrapancreatic $K_{ATP}$ channels with sulfonylureas are still unknown. The United Kingdom Prospective Diabetes Study (UKPDS), which followed patients treated with insulin, glibenclamide, or chlorpropamide, found no difference in mortality or diabetic end points between the different treatment groups.[23] However, the UKPDS was not specifically designed to compare the cardiovascular safety of different sulfonylureas, and the "glibenclamide-treated" group therefore included patients who had been subsequently transferred onto insulin therapy. A careful comparison is still required of cardiac morbidity in type 2 diabetic patients with ischemic heart disease treated with insulin, glibenclamide, or gliclazide.

## CONCLUSIONS

Studies on cloned $K_{ATP}$ channels have demonstrated that gliclazide and tolbutamide are specific inhibitors of $K_{ATP}$ channels containing SUR1, such as those found in pancreatic β cells, whereas other sulfonylureas like glibenclamide and glimepiride cross-react with $K_{ATP}$ channels from extrapancreatic tissues. It is therefore clear that the sulfonylureas which are widely prescribed for the treatment of type 2 diabetes should not be considered as a homogeneous group in terms of affinity and specificity for β-cell and extrapancreatic $K_{ATP}$ channels.

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