

## Changes in Recombinant ATP-sensitive $K^+$ Currents by Taurine Expressed in *Xenopus* Oocytes

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**Abstract :** ATP-sensitive potassium ( $K_{ATP}$ ) channels in different tissues usually share a common pore-forming Kir6.2 subunit, but possess different types of SUR subunit.  $K_{ATP}$  channels in the beta cell are composed of Kir6.2/SUR1, the skeletal and cardiac type of Kir6.2/SUR2A, and the vascular smooth muscle type of Kir6.2/SUR2B. We have compared the effect of taurine, which is a sulfonic aminoacid that is present particularly in mammalian excitable tissues at high concentrations, on the activities of  $K_{ATP}$  channels with distinct SUR subunits and their sulfonylurea sensitivities, using recombinant  $K_{ATP}$  channels expressed in *Xenopus laevis* oocytes. Intracellular taurine inhibited all the above three types of  $K_{ATP}$  channels in dose-dependent manners. They were well fit to the Hill equation with similar potencies, showing  $IC_{50}$  (50% inhibitory concentration of the channel activity) of  $10.6 \pm 1.34$  mM for Kir6.2/SUR1,  $12.3 \pm 1.9$  mM for Kir6.2/SUR2A, and  $12.6 \pm 1.3$  mM for Kir6.2/SUR2B. The Hill coefficients (h) were 4.5, 3.9, and 4.5, respectively. However, the taurine inhibition did not appear for Kir6.2 $\Delta$ C36 current. In the presence of 10 mmol/L taurine, the sensitivities of the three channel types to glibenclamide were enhanced to a similar extent compared to those in the absence of taurine. Sensitivity of the channels to gliclazide, another sulfonylurea, was not increased in the presence of taurine. From the above results, it might be speculated that the inhibitory effect of taurine on  $K_{ATP}$  channel activity is not related with different SUR subunits. The binding sites for glibenclamide on SURs, but not Kir6.2, might be critical for the taurine action.

**Key Words :** ATP-sensitive potassium channels, Glibenclamide, Gliclazide, Taurine, *Xenopus laevis* oocytes

## Introduction

Taurine (2-amino ethanesulfonic acid) is a low-molecular-weight organic cellular constituent that is found in considerable amounts in algae, animal kingdom but absent in bacterial and plant kingdom. It is ubiquitously, present in human tissues particularly at high concentrations in excitable and secretory cells [1]. Taurine has been known to play important roles in cellular osmoregulation [2-4],  $\text{Ca}^{2+}$  modulation [5,6], antioxidation [7] and radioprotection [8]. It is characterized by a sulfur-containing zwitterionic-amino acid in normal physiological pH with high hydrophilicity. Higher water solubility thus helps to act as an important organic osmolyte. Taurine readily interacts with neutral phospholipids [9] with both positive and negative charges in their molecules, thereby modifying the functions of membrane proteins embedded in, such as ion channels [10,11], membrane-associated proteins [12,13] as well as those in the membranes of intracellular organelles [14]. It reveals that taurine inhibits the ATP-sensitive potassium ( $\text{K}_{\text{ATP}}$ ) channels in cardiac [15,16] and skeletal muscles [17]. Ischaemic or hypoxic insults on the excitable cells could affect less by taurine efflux from the cells, thus relieving its inhibition of  $\text{K}_{\text{ATP}}$  channels [15,18]. This in turn hyperpolarizes the membrane potential and lets the cell consume less ATP. However, detailed molecular mechanism of the inhibition remains still unclear. Moreover, the taurine effect has not yet been evaluated on vascular smooth muscle-and pancreatic beta cell-type  $\text{K}_{\text{ATP}}$  channels.

The type of  $\text{K}_{\text{ATP}}$  channels differs in different tissues. The  $\text{K}_{\text{ATP}}$  channel is an octameric complex with 4 sulfonylurea receptor (SURx) subunits and 4 inwardly rectifying potassium channel (Kir6.2 or 6.1) subunits. Kir6.2/SUR1 is for the beta cell and some neurons [19,20], the cardiac and skeletal  $\text{K}_{\text{ATP}}$  channels of Kir6.2/SUR2A [21,22], and the smooth muscle and some neuronal  $\text{K}_{\text{ATP}}$  channels of Kir6.1 or

Kir6.2/SUR2B [23,24]. The Kir forms a pore while the SUR is a regulatory subunit that endows the Kir with sensitivity to drugs such as the inhibitory sulfonylureas [25] like glibenclamide and to  $\text{K}^{+}$  channel openers [26].

Recently, it is suggested that taurine increases the sensitivity of  $\text{K}_{\text{ATP}}$  channels on skeletal muscle cells, i.e. Kir6.2/SUR2A, to a sulfonylurea glibenclamide [17], implying taurine inhibits the  $\text{K}_{\text{ATP}}$  channel activity interfering with the glibenclamide-binding site of the channel. In contrast to SUR2A or 2B that has only a benzamido-binding site for glibenclamide, it is known that two cytoplasmic loops of SUR1 (one between transmembrane (TM) domain 15 and 16, and the other between TMs 5 and 6) are likely responsible for glibenclamide-binding, which lie in close proximity in the three-dimensional structure of the  $\text{K}_{\text{ATP}}$  channel [27]. The former loop may be shared for the sulfonylureas, such as tolbutamide, gliclazide, *etc.*, which have the sulfonylurea moiety only in the molecules. The latter loop between TMs 5 and 6, despite the residues unidentified as yet, may binds sulfonylureas with the benzamido moiety. Furthermore, it is not clear whether taurine acts on SUR, Kir6.2 or both. Using recombinant  $\text{K}_{\text{ATP}}$  channels expressed in *Xenopus* oocyte membrane, this study was undertaken to elucidate whether taurine inhibits all three types of  $\text{K}_{\text{ATP}}$  channels, and also whether there exists any difference in the potency according to the type of SURs. The molecular mechanism for the taurine action was also explored.

## Materials and Methods

### 1. Molecular biology

Mouse Kir6.2 (Genbank D50581; 20,28), rat SUR1 (Genbank L40624; 19), rat SUR2A (Genbank D83598; 21) and rat SUR2B (Genbank D86038; 23) cDNAs were cloned in the pBF vector. Mutagenesis of individual amino acids was performed using the altered

sites II System (Promega). Capped mRNA was prepared using the mMESSAGE mMACHINE large scale *in vitro* transcription kit (Ambion, Austin, TX, USA), as previously described [29].

## 2. Oocyte collection

Female *Xenopus laevis* were anaesthetised with MS222 (2 g/L added to the water). One ovary was removed via a mini-laparotomy, the incision was sutured and the animal was allowed to recover. Immature stage V-VI oocytes were incubated for 60 min with 1.0 mg/mL collagenase (type V, Sigma, USA) and manually defolliculated. Oocytes were then coinjected with about 0.1 ng of wild-type or mutant Kir6.2 mRNA and about 2 ng of mRNA encoding either SUR2A or SUR2B. The final injection volume was 50 nL/oocyte. Isolated oocytes were maintained in Barth's solution and studied for 1-4 days after the injection [29].

## 3. Electrophysiology

Patch pipettes were pulled from borosilicate glass and had resistances of 250-500 k $\Omega$  when filled with pipette solution. Macroscopic currents were recorded from giant excised inside-out patches at a holding potential of 0 mV and at 20-24 °C [29]. Currents were evoked by repetitive 3-second voltage ramps from -110 mV to +100 mV and recorded using a GeneClamp 500 patch-clamp amplifier (Axon Instruments, Foster, USA). Currents were filtered at 10 kHz, digitised at 0.4 kHz using a Digidata 1200 Interface and analysed using pClamp8.2 software (Axon Instruments, Foster, USA). Records were stored on videotape and resampled at 20 Hz for presentation in the figures.

The pipette (external) solution contained (mM): 140 KCl, 1.2 MgCl<sub>2</sub>, 2.6 CaCl<sub>2</sub>, 10 HEPES (pH 7.4 with KOH). The intracellular (bath) solution contained (mM): 107 KCl, 2 MgCl<sub>2</sub>, 1 CaCl<sub>2</sub>, 10 EGTA, 10

HEPES (pH 7.2 with KOH; final [K<sup>+</sup>] ~140 mM). Rapid exchange of solutions was achieved by positioning the patch in the mouth of one of a series of adjacent inflow pipes placed in the bath.

In ramp experiments, the slope conductance was measured by fitting a straight line to the current-voltage relation between -20 mV and -100 mV: the average of 5 consecutive ramps was calculated in each solution. The control current was taken as the mean of the current amplitude in control solution immediately before and after drugs application (averaged over 10 seconds in each case) except for glibenclamide irreversible on SUR1. Concentration-response curves were fit to the Hill equation:  $G/G_c = 1 / (1 + ([\text{drug}] / IC_{50})^h)$ , where [drug] is the drug concentration, IC<sub>50</sub> is the drug concentration at which inhibition is half-maximal, and h is the slope factor (Hill coefficient). All chemicals were purchased from Sigma Chemical Co. (St Louis, USA) except taurine (Tocris, Ellisville, USA). Drug-containing bath solutions were prepared just before the experiment. Glibenclamide and gliclazide were prepared as 10 and 50 mM stock solutions, respectively in DMSO and diluted immediately before use to the final concentrations indicated. Data were fit using Microcal Origin software and are presented as mean  $\pm$  SEM.

## Results

### 1. Direct effect of taurine on the recombinant K<sub>ATP</sub> channel currents expressed in the oocyte membrane

The taurine effect on the activities of different types of K<sub>ATP</sub> channels was explored in the inside-out mode. When applied intracellularly, taurine inhibited K<sub>ATP</sub> channel activity in a dose-dependent manner (Fig. 1 & 2). An IC<sub>50</sub> was 10.6  $\pm$  1.34 mM for Kir6.2/SUR1 channel, 12.3  $\pm$  1.9 mM for Kir6.2/SUR2A, and 12.6

$\pm 1.3$  mM for Kir6.2/SUR2B, indicating that the extent of inhibitory potency was quite similar. The Hill coefficients (*h*) were 4.5, 3.9, and 4.5, respectively. To examine the site on which taurine acts, taurine (15 mM) was applied onto Kir6.2 $\Delta$ C36 channel [30], a truncated form of Kir6.2 in which the last 36 amino acids of the C terminus had been deleted (Fig. 3). As expected, the channel current of Kir6.2  $\Delta$ C36 did not respond to the taurine application, implying the site of taurine being on the SUR subunits, not Kir6.2 subunit.

## 2. Effect of taurine on glibenclamide sensitivity of the $K_{ATP}$ channels

In order to elucidate whether taurine acts on the SURs interfering with the glibenclamide-binding sites, the channels were exposed to serial concentrations of glibenclamide in the presence of 10 mM taurine. In the presence of taurine, the glibenclamide sensitivities of all three recombinant  $K_{ATP}$  channels were markedly enhanced than those previously reported in the absence of taurine (31,32,33) (Fig. 4). They were fit to the Hill equation and yielded the  $IC_{50}$ s of  $0.3 \pm 0.05$  mM for Kir6.2/SUR1,  $3.7 \pm 0.67$  mM for Kir6.2/SUR2A, and  $2.6 \pm 0.98$  mM for Kir6.2/SUR2B. The Hill coefficients were  $0.3 \pm 0.02$ ,  $0.6 \pm 0.05$ , and  $0.3 \pm 0.05$ , respectively (Fig. 5).

## 3. Interference of taurine with both glibenclamide-binding sites on SUR1

As previously described, glibenclamide would bind two-binding sites on SUR1 as opposed to SUR2A or 2B, which has only the benzamido-binding site for glibenclamide. Gliclazide, another drug of sulfonylurea that binds only the sulfonylurea-, but not benzamido-binding site on SUR1, was further tested to ascertain the sites on SUR1 interacting with taurine. Gliclazide with taurine (10 mM), not as in the case of glibenclamide, similarly inhibited the Kir6.2/SUR1

channel compared to that in the absence of taurine (Fig. 6A), suggesting that taurine interacts with only sulfonylurea binding sites on SUR1. The  $IC_{50}$  was  $2.9 \pm 1.47$  mM, and the Hill coefficient was  $0.5 \pm 0.11$  (Fig. 6B).

## Discussion

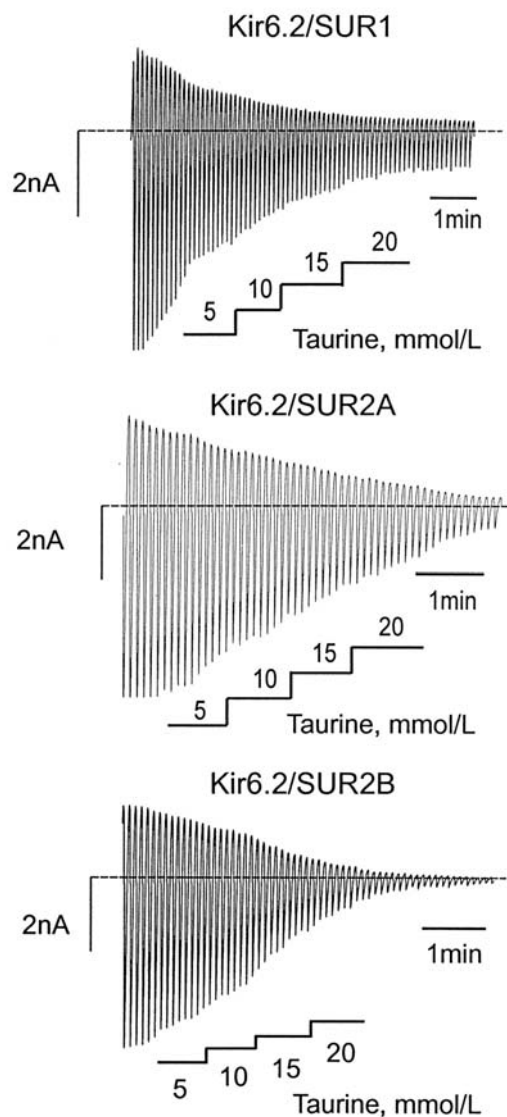
In the present study, we compared the taurine effects on the activities of the three-types of  $K_{ATP}$  channels, and demonstrated that taurine nearly equally inhibited the currents of Kir6.2/SUR1, Kir6.2/SUR2A, and Kir6.2/SUR2B channels, suggesting that the types of SUR subunits are not critical for the inhibitory mechanism of taurine. This suggestion was further supported by the fact that the glibenclamide sensitivities of the recombinant  $K_{ATP}$  channels were also equally accentuated in the presence of taurine. Since the effect of taurine was not observed in Kir6.2 $\Delta$ C36, but shown with the glibenclamide sensitivity of the channels, taurine may interact with SURs, in particular, their glibenclamide-binding sites, but not Kir6.2.

A recent study revealed that two nonadjacent cytosolic loops of SUR1, linking TMs 5 and 6 and TMs 15 and 16, are critical for [ $^3$ H]-glibenclamide binding [34]. Mutation of serine (S) 1237 in the cytoplasmic loop between TMs 15 and 16 to tyrosine (Y) abolishes the high-affinity block of Kir6.2/SUR1 channels by tolbutamide [35], which has only the sulfonylurea moiety in its molecule. It is likely that this residue also interacts with the sulfonylurea moiety of drugs such as glibenclamide, glimepiride, and gliclazide. The fact that glibenclamide blocks Kir6.2/SUR1-S1237Y channels indicates that residues other than S1237 are also critical for binding of this drug. Likewise, the block of Kir6.2/SUR1 by meglitinide and repaglinide, which do not possess a sulfonylurea moiety, is unaltered by the S1237Y mutation [35,36], suggesting that these drugs do not interact with this residue. This raises the

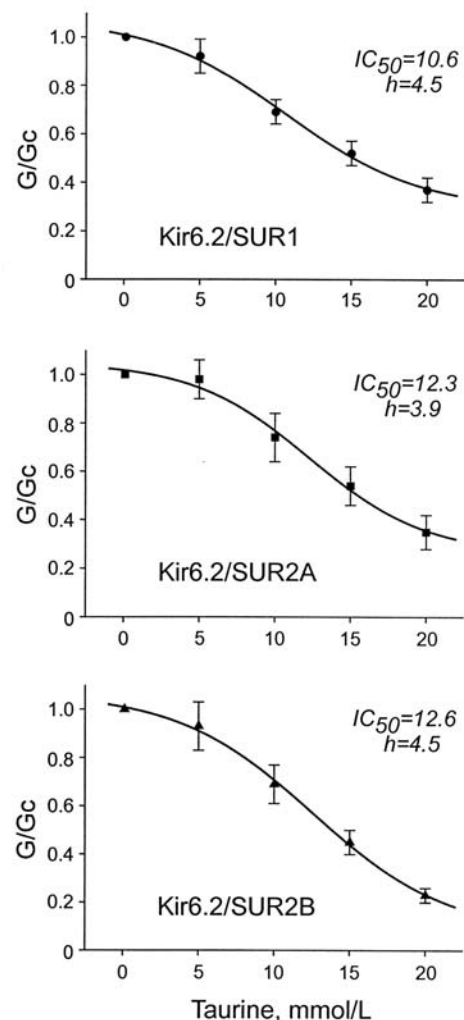
possibility that the TM 5-6 loop may interact with the benzamido moiety of glibenclamide. Because SUR2 has only the benzamido-binding site, glibenclamide could bind to SUR2 at one site, in contrast to SUR1 at two sites. Gliclazide, which has only the sulfonylurea moiety in its molecule, may bind to SUR1 at only the

sulfonylurea site, whereas SUR2 would not bind the drug. In the present study, taurine was found to contribute to the sensitization of Kir6.2/SUR1 channels to glibenclamide, but not gliclazide. This suggests that taurine interacts only with the benzamido-binding site and the interaction with only one binding site could be enough for the taurine action.

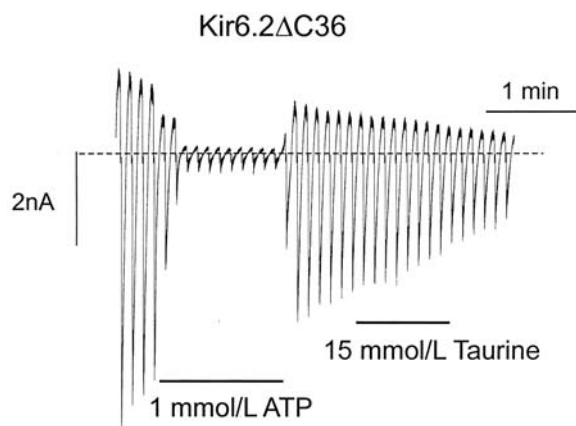
Taurine affects many different ion channels in cell membranes, including the Na<sup>+</sup>, Ca<sup>2+</sup>, Cl<sup>-</sup>, and K<sup>+</sup> channels. The underlying molecular mechanism seems



**Fig. 1.** Macroscopic currents recorded from inside-out patches in response to a series of voltage ramps from -110 to +100 mV. Oocytes were injected with mRNAs encoding Kir6.2/SUR1 (upper), Kir6.2/SUR2A (middle), or Kir6.2/SUR2B (lower). Taurine was applied at the bars indicated.



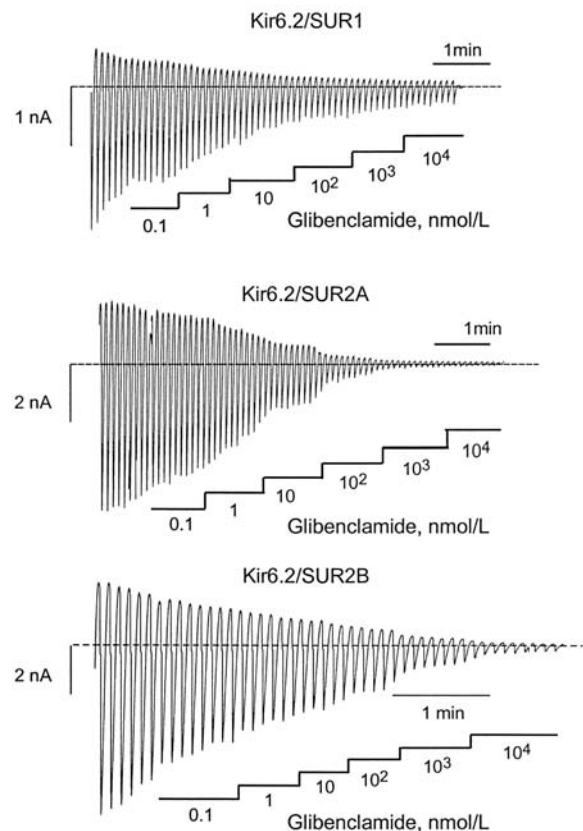
**Fig. 2.** Mean taurine dose-response relationships for Kir6.2/SUR1 (upper; n=5), Kir6.2/SUR2A (middle; n=5), or Kir6.2/SUR2B (lower; n=6) currents. Data represent Mean  $\pm$  SE.



**Fig. 3.** A macroscopic current recorded from inside-out patches in response to a series of voltage ramps from -110 to +100 mV. Oocytes were injected with mRNAs encoding Kir6.2  $\Delta$ C36. Taurine or ATP was applied at the bars indicated.

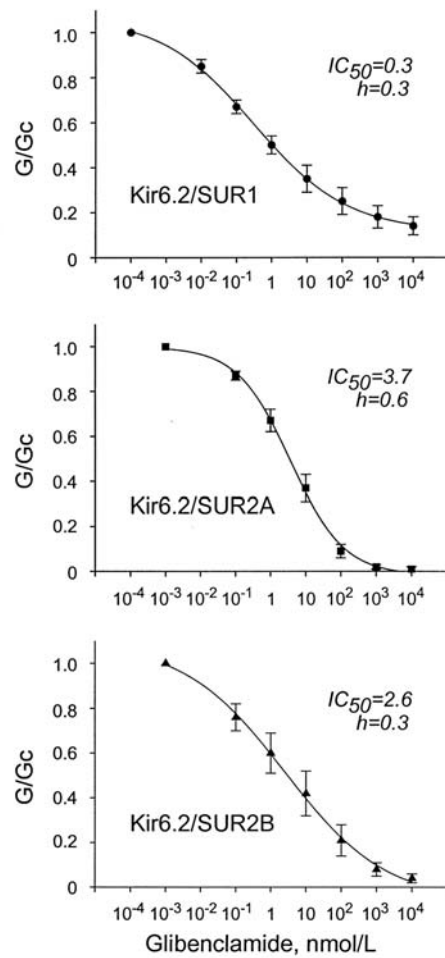
to be related with its zwitterionic nature similar to neutral membrane phospholipids, such as phosphatidylcholine and phosphatidylethanolamine [9]. It also interacts with phosphatidylserine, an acidic phospholipid, rendering higher  $\text{Ca}^{2+}$ -binding affinity. High-affinity taurine-binding sites have been identified on  $\text{Na}^+$ -dependent and -independent taurine transporters which are associated with taurine influx [37] and efflux [38], respectively. Low-affinity taurine binding was mainly observed with the neutral membrane phospholipids [9], possibly altering membrane architecture and fluidity. In skeletal muscle fibers, the inhibitory effect of taurine on  $\text{K}_{\text{ATP}}$  channel activity is independent of the functional state of the channel [17], supporting the notion that taurine may allosterically modify the  $\text{K}_{\text{ATP}}$  channel activity by binding to the polar phase of the membrane phospholipids that are functionally related to the SUR, but not SUR itself.

$\text{K}_{\text{ATP}}$  channels, which couple cellular metabolism to membrane excitability, are found at high density in a variety of cell types, including the pancreatic beta cells, cardiac, smooth, and skeletal muscles, and some brain neurons. In all these tissues, opening of  $\text{K}_{\text{ATP}}$  channels in



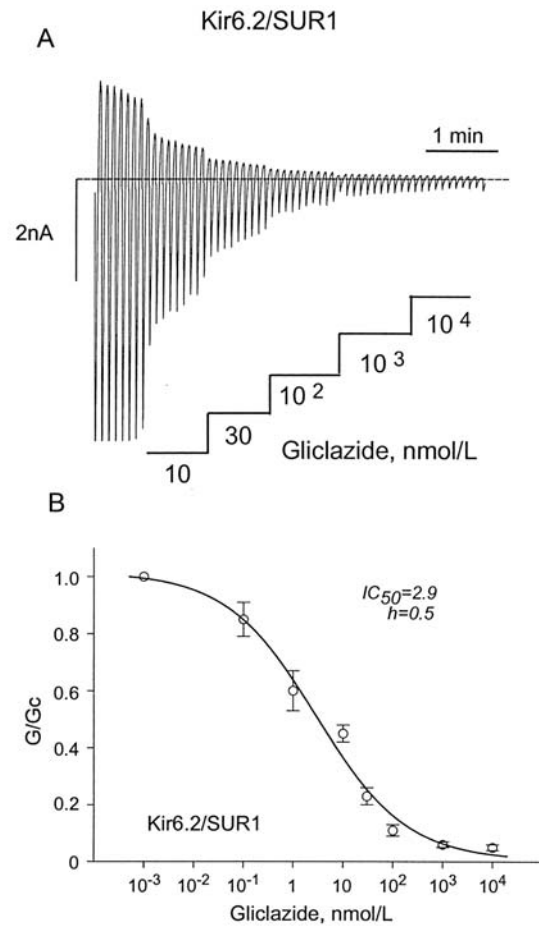
**Fig. 4.** Macroscopic currents recorded from inside-out patches in response to a series of voltage ramps from -110 to +100 mV. Oocytes were injected with mRNAs encoding Kir6.2/SUR1 (upper), Kir6.2/SUR2A (middle), or Kir6.2/SUR2B (lower). Glibenclamide was applied at the bars indicated.

response to metabolic stress leads to inhibition of electrical activity. Thus, when muscular or neuronal cells are exposed to ischaemia or hypoxia, taurine moves out of the cells [39], relieving its inhibition of  $\text{K}_{\text{ATP}}$  channels. This can induce an earlier activation of  $\text{K}_{\text{ATP}}$  channel activity to save cellular energy [15,16,17].  $\text{K}_{\text{ATP}}$  channels also play important roles in neuronal regulation of glucose homeostasis [40], seizure protection [41], and control of vascular smooth muscle tone [42]. In the beta cells, inhibited by cellular ATP and sulfonylureas,  $\text{K}_{\text{ATP}}$  channels are critical for the glucose-induced insulin secretion. The fact that taurine



**Fig. 5.** Mean glibenclamide dose-response relationships for Kir6.2/SUR1 (upper; n=5), Kir6.2/SUR2A (middle; n=7), or Kir6.2/SUR2B (lower; n=5) currents. Data represent Mean  $\pm$  SE.

inhibits all types of K<sub>ATP</sub> channels tested to a similar extent suggests that it plays important roles in the regulation of cellular functions linked to K<sub>ATP</sub> channel activity. It is well known that taurine is readily moved out of the cell as an osmolyte to prevent cell swelling during hypoosmotic insults. Furthermore, the concentrations of taurine are different among tissues and species; for example, 10-100  $\mu$ mol/L in serum, 30  $\mu$ mol/L/g wet wt in the rat heart, 52  $\mu$ mol/L/g dry wt in the pancreatic islets, 26  $\mu$ mol/L/g dry wt in the exocrine pancreas, 25.6  $\mu$ mol/L/g dry wt in the bovine cerebral



**Fig. 6.** A. A macroscopic current recorded from inside-out patches in response to a series of voltage ramps from -110 to +100 mV. Oocytes were injected with mRNAs encoding Kir6.2/SUR1. Gliclazide was applied at the bars indicated. B. Mean gliclazide dose-response relationships for Kir6.2/SUR1 (n=4) currents. Data represent Mean  $\pm$  SE.

cortex, 60  $\mu$ mol/L/g dry wt in the brain secretory structure [1,43]. Since the difference of SUR subunits could not modify the taurine inhibition of K<sub>ATP</sub> channel activity, intracellular endogenous taurine concentration seems to be a critical factor to regulate K<sub>ATP</sub> channel activity.

In conclusion, the inhibitory effect of taurine on K<sub>ATP</sub> channel activity is not related with different SUR subunits, but rather with intracellular concentration of

taurine. Taurine may interfere with the benzamido-binding site on SURs, but not Kir6.2 of  $K_{ATP}$  channel.

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