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Taurine block of cloned ATP-sensitive K^+ channels with different sulfonylurea receptor subunits expressed in *Xenopus laevis* oocytes

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Abstract

Taurine has been found to inhibit ATP-sensitive K^+ (K_{ATP}) channels in rat pancreatic beta-cells [Park et al., Biochem Pharmacol 2004;67:1089–1096] which could be due to its interaction with a benzamido-binding site on SUR1. In present study, we further evaluated the mechanism of taurine action on the K_{ATP}-channel inhibition, using cloned K_{ATP}-channels with different types of SUR subunit expressed in *Xenopus laevis* oocytes. The oocytes were coinjected with Kir6.2 mRNA, and mRNA encoding SUR1, SUR2A or SUR2B. Macroscopic currents were recorded from giant excised inside-out patches. The binding of glibenclamide to SUR1 was assessed by using a glibenclamide-fluorescent probe. Intracellular taurine inhibited all three types of K_{ATP}-channels to a similar extent. They were fit to the Hill equation, showing IC_{50} of 11.0 mM for Kir6.2/SUR1, 10.9 mM for Kir6.2/SUR2A, and 9.0 mM for Kir6.2/SUR2B currents. Taurine at the concentration of 10 mM enhanced the high-affinity bindings of glibenclamide and repaglinide on all types of SUR, whereas the low-affinity binding on Kir6.2 was not affected. The intensity of glibenclamide fluorescence was higher in the plasma membrane of taurine-pretreated oocytes. The high-affinity binding of tolbutamide or gliclazide on SUR was not modified by taurine. These results suggest that the taurine inhibition of K_{ATP}-channels is mediated by an interaction with the site on SUR where the benzamido group is bound. Therefore, intracellular concentrations of taurine in different tissues may be more important in determining taurine modulation of the K_{ATP}-channel rather than distinct types of SUR subunit.

Keywords: Taurine; K_{ATP}-channel; Sulfonylurea; *Xenopus* oocyte; Kir6.2; SUR

1. Introduction

Taurine (2-amino ethanesulfonic acid) is a sulfonic amino acid that is present at considerable amounts in algae and animal kingdom but absent in bacterial and plant kingdom [1]. Studies have shown that in both excitable and nonexcitable tissues taurine can modulate cell function through its effect on ion channel activity [2]. This regulation may be attributable to the interaction of taurine with neutral membrane phospholipids [3] that have also positive and negative charges in their molecules, thereby modifying the functions of ion channels [4–7]. As a medical supplement, taurine has been effective for diabetes, skeletal muscle disorder [8], and heart failure [7].

The activity of K_{ATP}-channels in pancreatic [9], cardiac [7,10] and skeletal muscles [11] has been reported to be influenced by taurine. The K_{ATP}-channel is an octameric complex consisting of regulatory tetramer of SUR subunits plus pore-forming tetramer of Kir6.2 subunits. But the type of K_{ATP}-channel can be distinct in different tissues: Kir6.2/SUR1 for beta-cells and some neurons [12,13],

Abbreviations: *G*, conductance; *h*, Hill coefficient; K_{ATP}-channel, ATP-sensitive potassium channel; SUR, sulfonylurea receptor; Kir, inwardly-rectifying potassium channel; TM, transmembrane domains; ATP, adenosine triphosphate; ADP, adenosine diphosphate; GES, guanidinoethane sulfonate; *N*, number of observations

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cardiac and skeletal K_{ATP}-channels of Kir6.2/SUR2A [14,15], and vascular smooth muscle K_{ATP}-channels of Kir6.2/SUR2B [16]. Normal intracellular taurine concentration varies ranging 10–60 mM among tissues where different membrane-associated intracellular modulators for K_{ATP}-channels may exist [1,17]. Taurine is thought to be one of intracellular inhibitors of the K_{ATP}-channel. The activity of K_{ATP}-channels could be also affected by tissue specificity in the different membrane fluidity and architecture among the native cells [3]. Therefore, it is necessary to use a common condition, such as an oocyte expression system, for better understanding of the taurine action on the diverse types of K_{ATP}-channels.

Very recently, we have reported that taurine inhibits native beta-cell K_{ATP}-channels, at least in part, through an interaction with a benzamido-binding site for glucose-lowering sulfonylureas on SUR1 [9]. A sulfonylurea drug glibenclamide has two binding moieties; a sulfonylurea moiety and a benzamido moiety that can interact with the SUR subunit. Whereas tolbutamide and gliclazide have only the sulfonylurea moiety [18], repaglinide and meglitinide have only the benzamido moiety [19,20] (Fig. 1). SUR1 can bind both moieties of sulfonylureas, while SUR2A and SUR2B can only bind the benzamido moiety of sulfonylureas, probably because of the bulkier volume of the tyrosine side chain in the sulfonylurea-moiety-binding site on SUR2 [20], compared with that of serine present at the corresponding site on SUR1. These sulfonylurea bindings on SUR are known to be a high-affinity binding that occurs at sulfonylurea concentrations less than 10 μM [21]. It has been also assumed at sulfonylurea concentrations exceeding 10 μM that a second low-affinity binding of the drugs is associated with Kir6.2, or possibly Kir6.2 through a regulatory protein endogenous to *Xenopus* oocyte [22].

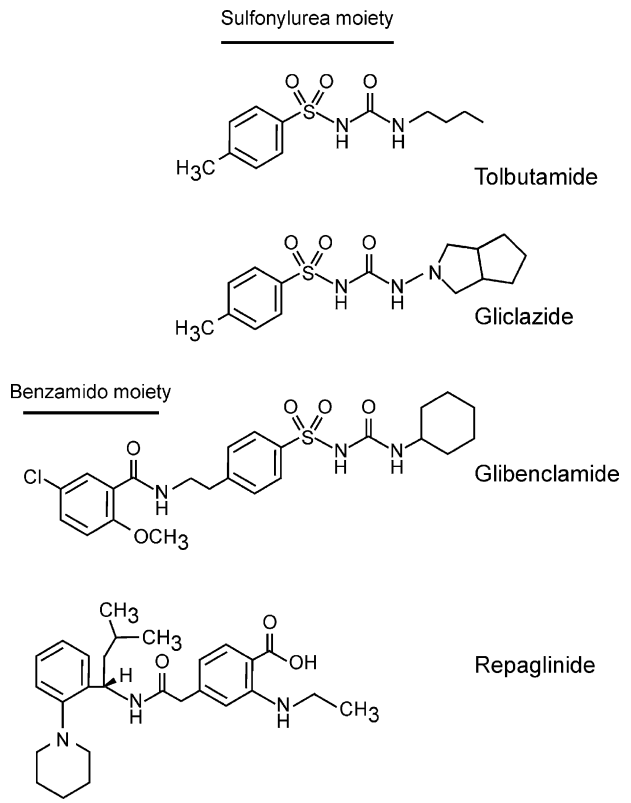

Fig. 1. Molecular structures of tolbutamide, gliclazide, glibenclamide, and repaglinide.

Using these proposed binding characteristics of the sulfonylurea drugs and cloned K_{ATP}-channels expressed in *Xenopus* oocyte membrane, we have compared the potency and the inhibitory mechanism of taurine on K_{ATP}-channels to evaluate whether different SUR types are involved in this taurine inhibition.

2. Materials and methods

2.1. Molecular biology

Mouse Kir6.2 (Genbank D50581 [13,23]), rat SUR1 (Genbank L40624 [12]), rat SUR2A (Genbank D83598 [14]) and rat SUR2B (Genbank D86038 [16]) cDNAs were cloned in the pBF vector which were kindly provided by Professor Ashcroft and co-workers [23]. Capped mRNA was prepared using the mMESSAGE mMACHINE large scale in vitro transcription kit (Ambion), as previously described [24].

2.2. Oocyte collection

Female *Xenopus laevis* were anaesthetized with MS222 (2 g/L added to the water). One ovary was removed via a mini-laparotomy, the incision sutured and the animal allowed to recover. Immature stage V–VI oocytes were incubated for 60 min with 1.0 mg/L collagenase (type V, Sigma) and manually defolliculated. Oocytes were coinjected with 0.1 ng of Kir6.2 mRNA and 2 ng of mRNA encoding SUR1, SUR2A or SUR2B (giving a 1:20 ratio). The final injection volume was 50 nL/oocyte. Isolated oocytes were maintained in Barth's solution at 19° and studied 1–4 days after injection [24].

2.3. Electrophysiology

Patch electrodes were pulled from thick-walled borosilicate glass (Harvard) and had resistances of 250–500 kΩ when filled with pipette solution. Macroscopic currents were recorded from giant excised inside-out patches at a holding potential of 0 mV. Currents were evoked by repetitive 3 s voltage ramps from −110 to +100 mV and recorded using a GeneClamp 500 patch-clamp amplifier (Axon Instruments). They were filtered at 10 kHz, digitized at 1 kHz using a Digidata 1200 Interface and analyzed using pClamp8.2 software (Axon). Records were stored on videotape and resampled at 20 Hz for presentation in the figures. The pipette (external) solution contained

the following (mM): 140 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, 10 HEPES (pH 7.4 with KOH). The intracellular (bath) solution contained (mM): 107 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 EGTA, 10 HEPES (pH 7.2 with KOH; final $[K^+] \sim$ 140 mM). All chemicals were purchased from Sigma Chemical Co. except taurine (Tocris) and repaglinide (kindly provided by Novo Nordisk Korea). Drug-containing bath solutions were made just before the experiment. Glibenclamide and repaglinide, and gliclazide were prepared as 10 and 50 mM stock solutions in dimethyl sulfoxide (DMSO), respectively and diluted as required. Tolbutamide was made up as a 0.1 M stock solution in 0.2 M KOH. 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. Experiments were carried out at room temperature (20–24°).

2.4. Data analysis

The slope conductance was measured by fitting a straight line to the current–voltage relation between −20 and −100 mV: the average of five consecutive ramps was calculated in each solution. To control for rundown, experiments started only when the currents did not have a significant rundown after ATP application (1 mM) and the control conductance was taken as the mean of that obtained in control solution before and after application of test compounds. The effect of glibenclamide and repaglinide was not obviously reversible, so each patch was exposed to only one concentration, and only one patch was tested per oocyte. According to the same reason, the conductance in the presence of glibenclamide and repaglinide was expressed relative to that in control solution before the drug application for both Kir6.2/SUR1 and Kir6.2/SUR2 currents.

The conductance (G) is plotted as a fraction of that obtained in the control solution (G_c). Concentration–response curves for glibenclamide were fit to the following equation [19]:

$$\frac{G}{G_c} = x \times y \tag{1}$$

$$x = L + \frac{(1-L)}{1 + ([\text{Drug}]/\text{IC}_{50(1)})^{h1}} \tag{2}$$

$$y = \frac{1}{1 + ([\text{Drug}]/\text{IC}_{50(2)})^{h2}} \tag{3}$$

where [Drug] is the glibenclamide concentration, $IC_{50(1)}$ and $IC_{50(2)}$ are the concentrations at which inhibition is half maximal at the high-affinity and low-affinity sites, respectively; $h1$ and $h2$ are the Hill coefficients (slope factors) for the high-affinity and low-affinity sites, respectively; and L is the fractional conductance remaining when all of the high-affinity inhibitory sites are occupied. As we did not consider low-affinity binding of gliclazide on Kir6.2,

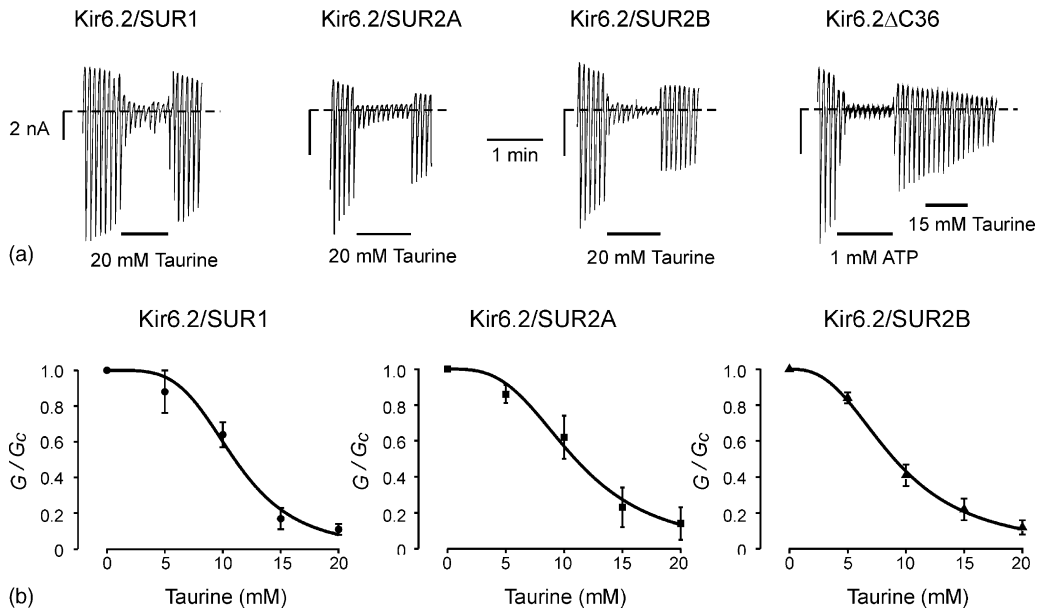

Fig. 2. Inhibition of K_{ATP} currents by taurine. (a) Macroscopic currents recorded from inside-out patches in response to a series of voltage ramps from −110 to +100 mV from oocytes coexpressing Kir6.2 and either SUR1, SUR2A or SUR2B. A macroscopic cloned Kir6.2ΔC36 current (fourth trace) cited from [9]. Taurine and ATP were added as indicated by the bars. The dashed line indicates the zero current level. (b) Concentration–inhibition relationships for block of K_{ATP} currents by taurine. Taurine concentration–response relationships for Kir6.2/SUR1 ($N = 7$–9), Kir6.2/SUR2A ($N = 12$–14) and Kir6.2/SUR2B ($N = 12$–14) currents. The macroscopic conductance in the presence of taurine (G) is expressed as a fraction of its mean amplitude obtained before and after exposure to taurine (G_c). The symbols represent the mean, and the vertical bars indicate 1 S.E.M. The lines are fit to Eq. (5) in the text using the following values. Kir6.2/SUR1 channels: $IC_{50} = 11.0$ mM, $h = 4.1$. Kir6.2/SUR2A channels: $IC_{50} = 10.9$ mM, $h = 3.1$. Kir6.2/SUR2B channels: $IC_{50} = 9.0$ mM, $h = 2.6$.

gliclazide concentration–response curves were made by using Eq. (2).

$$\frac{G}{G_c} = x \tag{4}$$

For taurine concentration–response curves, the parameter names in Eq. (3) were changed.

$$\frac{G}{G_c} = \frac{1}{1 + ([\text{Taurine}]/\text{IC}_{50})^h} \tag{5}$$

where [Taurine] is the taurine concentration, IC_{50} the concentration at which inhibition is half maximal, h the Hill coefficient (slope factor). Data were fit using Microcal Origin Software and the symbols in the figures represent the mean, and the vertical bars indicate 1 S.E.M.

2.5. Confocal imaging of glibenclamide fluorescence

The binding of glibenclamide to SUR1 expressed in the membrane of *Xenopus* oocytes was assessed using the green fluorescent probe glibenclamide-BODIPY FL (Molecular Probes). Oocytes were stained with glibenclamide-BODIPY FL (1 nM) for 20–30 min at room temperature. The fluorescence of five oocytes per group was scanned from the cut surface to a depth of 10 μm using the LSM-510META confocal laser scanning microscope (Carl Zeiss). Fluorescence was measured using an excitation wavelength of 488 nm line of an argon laser, dichroic 505 nm long pass, and emitter bandpass of 535 nm with neutral density filters to attenuate the excitation light intensity. The relative intensity of the plasma membrane fluorescence was divided by the average relative intensity of the cytoplasmic fluorescence. For each group, 1–2 separate oocytes per group were included in the confocal imaging. Images were analyzed using LSM-510 META software Release 3.2 (Carl Zeiss) and the software program Simple32 (Compix Inc.).

2.6. Statistical analysis

Results are expressed as means ± S.E.M., and significance was determined by analysis with unpaired Student's *t*-test for two-group comparison or ANOVA for multiple-group comparison. $P < 0.05$ was considered significant.

3. Results

3.1. Direct effect of taurine on cloned K_{ATP}-channel currents expressed in the oocyte membrane

We first compared the direct effect of taurine on Kir6.2/SUR1, Kir6.2/SUR2A, and Kir6.2/SUR2B currents. When applied intracellulary, taurine inhibited all three types of the cloned K_{ATP}-channel, whose inhibitions were reversible (Fig. 2). We have previously shown that taurine does not inhibit the current of Kir6.2ΔC36 channel (fourth trace in Fig. 2a from [9]), a truncated form of Kir6.2 in which the last 36 amino acids of the C-terminus were deleted and therefore it could be expressed in the membrane without

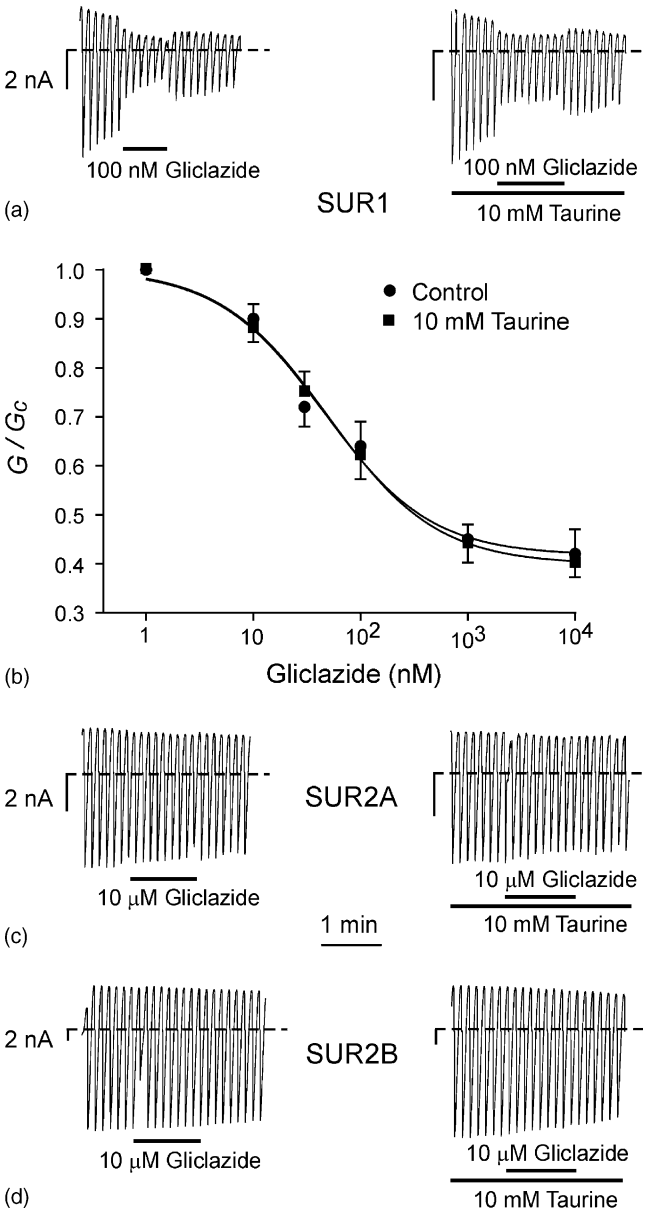

Fig. 3. Block of cloned K_{ATP} currents by gliclazide. Macroscopic currents recorded from inside-out patches in response to a series of voltage ramps from −110 to +100 mV from oocytes coexpressing Kir6.2 and either SUR1 (a), SUR2A (c) or SUR2B (d) in the absence (left) and presence (right) of taurine (10 mM). Taurine and gliclazide were added as indicated by the bars. The dashed line indicates the zero current level. (b) High-affinity gliclazide concentration–responses for Kir6.2/SUR1 currents in the absence (circles, $N = 4$–10) and presence (squares, $N = 4$–10) of taurine (10 mM). The macroscopic conductance in the presence of gliclazide (G) is expressed as a fraction of its mean amplitude obtained before and after exposure to the drug (G_c). The symbols represent the mean, and the vertical bars indicate 1 S.E.M. The lines are fit to Eq. (4) in the text using the following values. In the absence of taurine, $IC_{50} = 45.8$ nM, $h = 0.87$, $L = 0.42$; in the presence of taurine, $IC_{50} = 49.9$ nM, $h = 0.86$, $L = 0.39$.

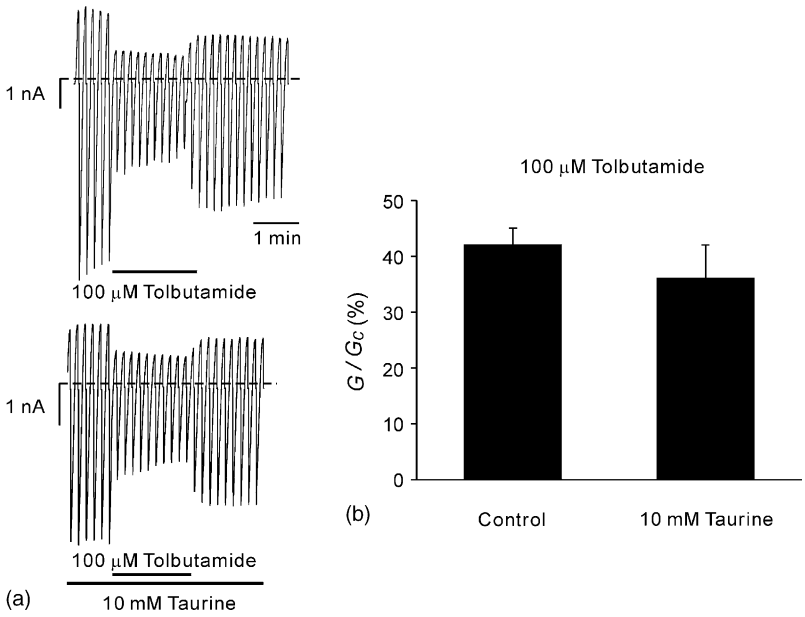

Fig. 4. Block of cloned K_{ATP} currents by tolbutamide. (a) Macroscopic currents recorded from inside-out patches in response to a series of voltage ramps from −110 to +100 mV from oocytes coexpressing Kir6.2 and SUR1 in the absence (upper) and presence (lower) of taurine (10 mM). Taurine and tolbutamide were added as indicated by the bars. The dashed line indicates the zero current level. (b) Mean amplitude of Kir6.2/SUR1 currents recorded in response to 100 μM tolbutamide, expressed as a percentage of the control current amplitude in the absence (left, $N = 8$) and presence (right, $N = 5$) of taurine (10 mM). The symbols represent the mean, and the vertical bars indicate 1 S.E.M.

SUR. It indicates that the inhibition of K_{ATP} current by taurine is attributable to a taurine interaction with SUR subunit, not Kir6.2 subunit. IC_{50} for taurine inhibition was 11.0 ± 0.57 mM for Kir6.2/SUR1 channel, 10.9 ± 0.55 mM for Kir6.2/SUR2A, and 9.0 ± 0.19 mM for Kir6.2/SUR2B (Fig. 2b), indicating no remarkable difference in the extent of taurine potency in K_{ATP}-channels with the different types of SUR subunit. The Hill coefficients (h) were 4.1 ± 0.83, 3.1 ± 0.48, and 2.6 ± 0.13, respectively.

3.2. Effect of taurine on gliclazide and tolbutamide sensitivity of cloned K_{ATP}-channels

In order to see which site on SUR is associated with the current inhibition of taurine, we tested possible taurine-mediated change in gliclazide sensitivity of cloned K_{ATP}-channels. Gliclazide and tolbutamide have only the sulfonylurea moiety within their own structure which can interact with SUR in the K_{ATP}-channel. As shown in Fig. 3a and b, gliclazide (100 nM) inhibited the Kir6.2/SUR1

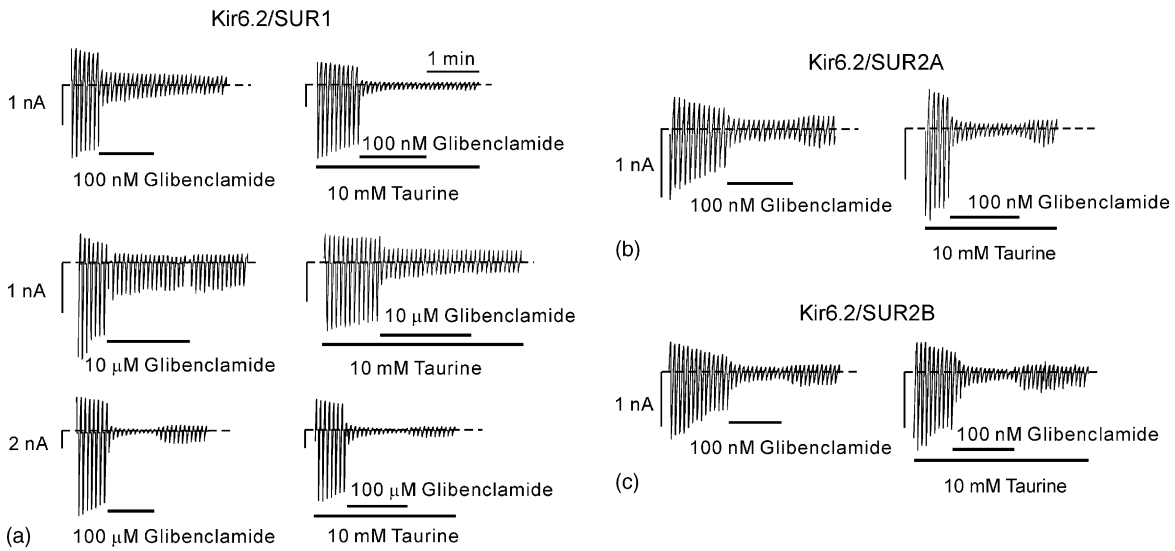

Fig. 5. Block of cloned K_{ATP} currents by glibenclamide. Macroscopic currents recorded from inside-out patches in response to a series of voltage ramps from −110 to +100 mV from oocytes coexpressing Kir6.2 and either SUR1 (a), SUR2A (b) or SUR2B (c) in the absence (left) and presence (right) of taurine (10 mM). Taurine and glibenclamide were added as indicated by the bars. The dashed line indicates the zero current level.

current to a similar extent in the presence or absence of 10 mM taurine. The residual currents at 100 nM of gliclazide were 64 ± 5% ($N = 6$) in the absence of taurine and 62 ± 5% ($N = 5$) in the presence of taurine. IC_{50} and the Hill coefficient for high-affinity binding of gliclazide were 45.8 ± 11.34 nM and 0.87 ± 0.18 in the control without taurine (50 nM in [25]); 49.88 ± 5.56 nM and 0.86 ± 0.08 in the presence of taurine. Kir6.2/SUR2A and Kir6.2/SUR2B currents were not inhibited by gliclazide in the range of <10 μM (Fig. 3c and d), consistent with the previous finding that SUR2A and SUR2B do not possess high-affinity binding sites for the sulfonylurea moiety [25]. Like the case of gliclazide, taurine did not induce an increase in the tolbutamide sensitivity of Kir6.2/SUR1 channel (Fig. 4). At 100 μM concentration of tolbutamide, the remaining Kir6.2/SUR1 currents were 42 ± 3% and 36 ± 6% in the absence and presence of taurine, respectively, which was insignificant by *t*-test.

3.3. Effect of taurine on glibenclamide sensitivity of cloned K_{ATP}-channels

We further explored the taurine interaction site on SUR using glibenclamide, which has two binding moieties for SUR subunits of the K_{ATP}-channel [26]. Unlike gliclazide or tolbutamide, glibenclamide could inhibit Kir6.2/SUR2A and Kir6.2/SUR2B currents (Fig. 5), implying that the benzamido moiety of glibenclamide can interact with SUR2A and SUR2B to block the channel. In the presence of taurine, the glibenclamide-mediated channel inhibition was remarkably enhanced in all three Kir6.2/SUR1, Kir6.2/SUR2A, and Kir6.2/SUR2B currents, but the taurine action was only effective in the range of ≤10 μM of glibenclamide, but not at 100 μM of glibenclamide (third traces in Fig. 5a). The double-Hill concentration–response curves for the glibenclamide block in the absence and presence of 10 mM taurine were obtained (Fig. 6), and the fitting parameters were summarized in Table 1. IC_{50} for high-affinity binding ($IC_{50(1)}$) in the presence of taurine was significantly less than that in the absence of taurine in all types of the

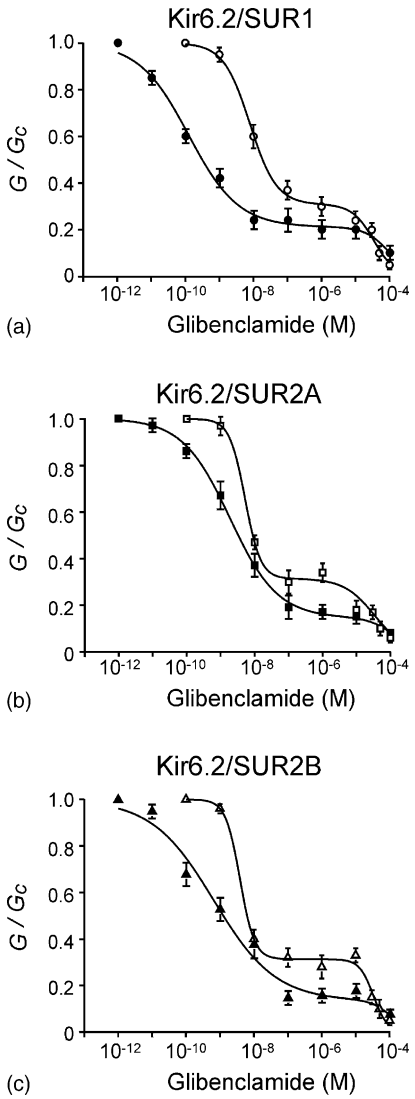

Fig. 6. Concentration–inhibition relationships for block of K_{ATP} currents by glibenclamide. glibenclamide concentration–response relationships in the absence (open circles) and presence (filled circle) of taurine (10 mM) for (a) Kir6.2/SUR1, (b) Kir6.2/SUR2A and (c) Kir6.2/SUR2B currents. $N = 4$–10. The macroscopic conductance (G) is expressed as a fraction of its mean amplitude obtained before exposure to the drug (G_c). The symbols represent the mean, and the vertical bars indicate 1 S.E.M. The lines are fit to Eq. (1) in the text using the values summarized in Table 1.

Table 1
Taurine modification of the IC_{50} for K_{ATP} channel inhibition by glibenclamide

	SUR1, control	SUR2A		SUR2B		Taurine
		Taurine	Control	Taurine	Control	
High-affinity						
L	0.31 ± 0.02	0.21 ± 0.03	0.31 ± 0.04	0.14 ± 0.01	0.31 ± 0.02	0.14 ± 0.08
$IC_{50(1)}$ (nM)	7.90 ± 1.17	0.12 ± 0.03*	5.20 ± 0.99	1.90 ± 0.22*	3.89 ± 0.84	0.65 ± 0.45*
*h*1	1.12 ± 0.19	0.6 ± 0.08	1.89 ± 0.96	0.60 ± 0.04	2.05 ± 0.41	0.50 ± 0.12
Low-affinity						
$IC_{50(2)}$ (μM)	55 ± 21	93 ± 53	53 ± 30	122 ± 54	52 ± 28	110 ± 60
*h*2	1.38 ± 0.39	1.30 ± 1.6	0.97 ± 0.65	1.20 ± 1.6	2.11 ± 0.74	1.50 ± 1.1

Values are means ± S.E.M. ($N = 4$–10).
* $P < 0.05$ compared to IC_{50} measured in the absence of taurine (control) in each SUR group.

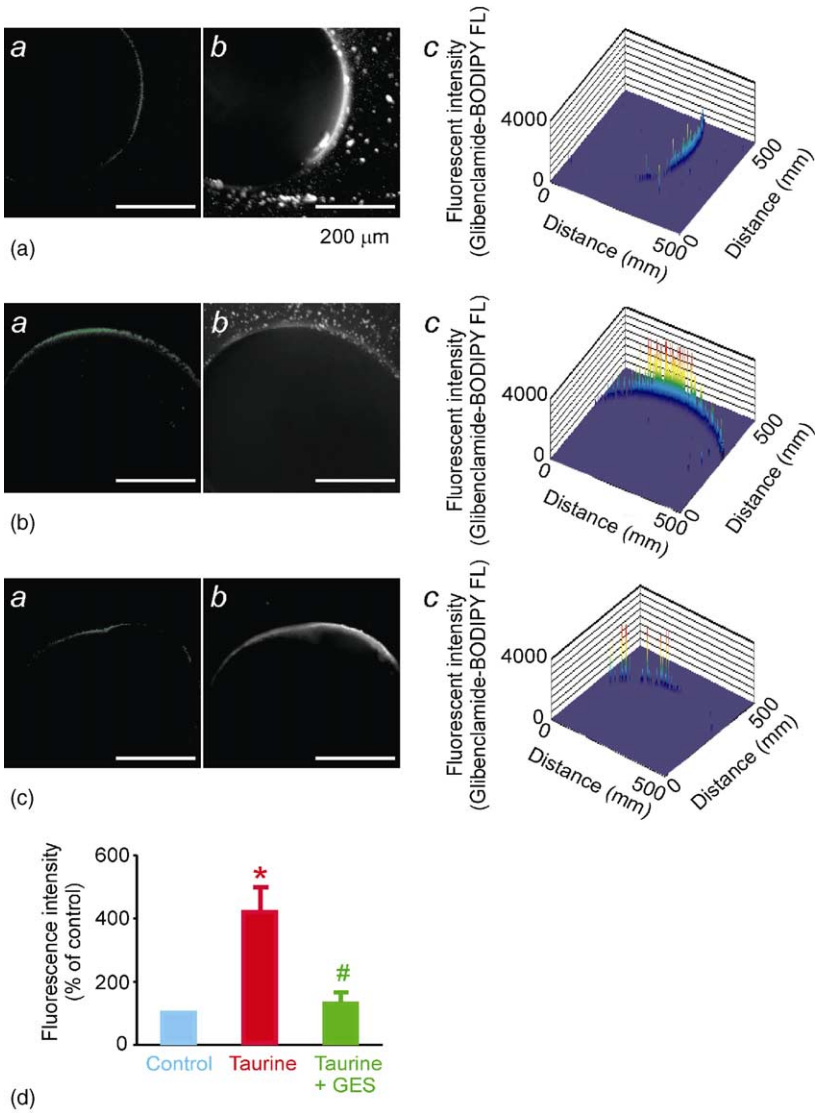

Fig. 7. Comparison of the glibenclamide binding to SUR1 by confocal laser scanning microscope. Confocal laser scanning microscopy was performed for *Xenopus* oocytes expressing Kir6.2 and SUR1. The SUR-binding green fluorescent probe (glibenclamide-BODIPY FL)-stained oocytes visualized under fluorescent microscopy (a) and combination of transmitted light with differential interference contrast optics (b). Fluorescence intensity was measured in each individual image. Pseudo three-dimensional profile of images shown in Fig. 8a–c (c). Original magnification, 100×. Oocytes were randomly selected. Confocal images are representative in control (a), taurine-pretreated (b) and taurine + GES-pretreated (c) oocytes. The graph displays the fluorescence intensity (expressed as a percentage of control) from control (sky blue column, $N = 7$), taurine-pretreated oocytes (orange column, $N = 6$) and taurine + GES-pretreated oocytes (green column, $N = 5$) groups (d). $^{*}P < 0.05$ compared to control; $^{\#}P < 0.05$ compared to taurine-pretreated oocytes group.

K_{ATP}-channel (0.12 ± 0.03 versus 7.9 ± 1.17 nM for Kir6.2/SUR1; 1.9 ± 0.22 versus 5.2 ± 0.99 nM for Kir6.2/SUR2A; 0.65 ± 0.45 versus 3.89 ± 0.84 nM for Kir6.2/SUR2B currents). Though the low-affinity site was also apparent from the glibenclamide concentration–response curves, IC_{50} for low-affinity binding ($IC_{50(2)}$) in the presence of taurine was rather greater, but not significantly different from the result in the absence of taurine.

3.4. Effect of taurine on the binding of glibenclamide to SUR1

Xenopus oocytes expressing Kir6.2 and SUR1 were visualized under a confocal laser scanning microscope after staining with the SUR-binding green fluorescent probe glibenclamide-BODIPY FL (Fig. 7a–c). Pseudo three-dimensional images (Fig. 7a–c, 7b–c, and 7c–c) demonstrated that the glibenclamide signal localized almost exclusively to the plasma membrane. No loci of enhanced fluorescence could be detected within oocytes. To determine whether taurine affects the binding of glibenclamide to SUR1 subunit, we compared the fluorescent intensity in the plasma membrane between control (Fig. 7a) and taurine-pretreated (0.3 mM for over 3 h) (Fig. 7b) oocytes. The ratio of plasma membrane to cytoplasmic fluorescence intensity was significantly higher in taurine-pretreated oocytes than in control oocytes. However, taurine-induced increase in the ratio was significantly

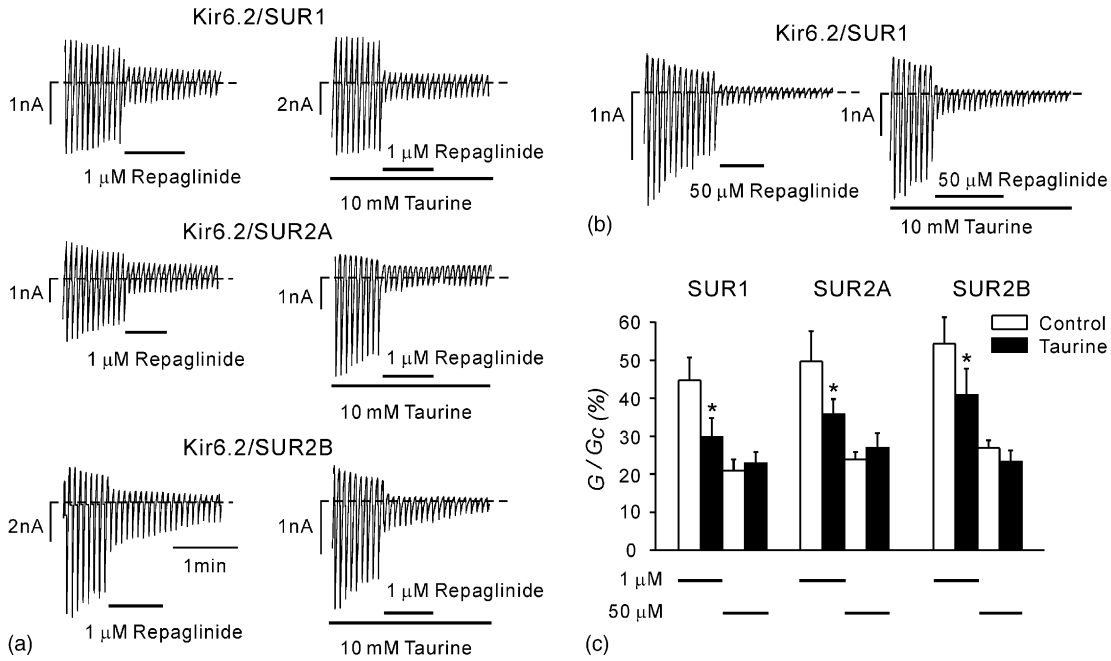

Fig. 8. Block of cloned K_{ATP} currents by repaglinide. (a) Macroscopic currents recorded from inside-out patches in response to a series of voltage ramps from −110 to +100 mV from oocytes coexpressing Kir6.2 and either SUR1 (upper), SUR2A (middle) or SUR2B (lower) in the absence (left) and presence (right) of taurine (10 mM). Taurine and repaglinide were added as indicated by the bars. The dashed line indicates the zero current level. (b) Mean amplitude of K_{ATP} currents recorded in response to 1 or 50 μM of repaglinide, expressed as a percentage of the control current amplitude in the absence (open bars) and presence (filled bars) of taurine (10 mM). The symbols represent the mean, and the vertical bars indicate 1 S.E.M. ($N = 4$–6). $^{*}P < 0.05$ compared to control obtained at the same concentration of repaglinide in each SUR group.

decreased by GES, a Na^+-dependent taurine transporter inhibitor, which was pretreated just prior to taurine application (Fig. 7c). The percent increases in the ratio were 420 ± 75 for taurine-pretreated oocytes ($N = 6$) and 130 ± 40% for taurine + GES-pretreated oocytes ($N = 5$) compared with control ($N = 7$) (Fig. 7d).

3.5. Effect of taurine on repaglinide sensitivity of cloned K_{ATP}-channels

To elucidate the taurine-mediated increase in glibenclamide sensitivity of the K_{ATP}-channel being associated with the benzamido-binding site on SUR, we tested another sulfonylurea repaglinide that has only the benzamido moiety to interact with SUR. At the concentrations less than 10 μM, the repaglinide-induced current block was significantly enhanced in the presence of taurine in all types of K_{ATP}-channels (Fig. 8a and c). However, taurine did not show any significant effect on the channel sensitivity in response to 50 μM repaglinide (Fig. 8b and c), suggesting that the mechanism for taurine-mediated increase in glibenclamide sensitivity of the K_{ATP}-channel is also critical for the case of repaglinide.

4. Discussion

As regulatory subunits of K_{ATP}-channels, SUR1 and SUR2 have ∼60% homology at the amino acid level and have similar predicted membrane topologies [27]. However, when they form K_{ATP}-channels with Kir6.2, they exhibit many different functional aspects. For example, various stimulators of K_{ATP}-channel activity, which mediate their effects by interaction with SUR, show distinct efficacies according to the types of SUR [21]. Diazoxide, a K_{ATP}-channel opener, markedly activates beta-cell type Kir6.2/SUR1 currents but it does not affect striated muscle-type Kir6.2/SUR2A currents, while the reverse is true for another opener pinacidil. Although SUR2A and SUR2B are splice variants of the same gene that differ only in their C-terminal 42–45 amino acids [28], Kir6.2/SUR2A and Kir6.2/SUR2B channels respond differently to nucleotides and the K_{ATP}-channel opener pinacidil [29]. Glibenclamide, glimepiride and repaglinide, of which effect is also mediated by SUR, block all three types of K_{ATP}-channels. The former two drugs are hardly reversible specifically in Kir6.2/SUR1 [30], whereas repaglinide shows a poor reversibility in all types of K_{ATP} currents [19]. However, mitiglinide, tolbutamide and gliclazide have the beta-cell K_{ATP}-channel selectivity [20].

In the case of taurine, we demonstrated that taurine inhibited the different types of K_{ATP}-channels with a similar potency when expressed in the same oocyte system. IC_{50} for Kir6.2/SUR1 channel was 11.0 mM, 10.9 mM for Kir6.2/SUR2A, and 9.0 mM for Kir6.2/SUR2B, consistent with those of native-beta-cells (12.3 mM [9]) and cardiac myocytes (13.5 mM [10]). But they are different from that of native skeletal myocytes

(20.1 mM [11]). This discrepancy with the latter finding may be explained by several mechanisms. First, splice variants of SUR2A possibly showing a different pharmacological profile can be expressed in native skeletal muscles. Second, multi-channel preparations in patch-clamp experiments might cause interactions between different channels, reducing the sensitivity of the channels to the blockers [11]. In addition, modulators associated with sarcolemma or its own characteristics of skeletal myocytes may be influential in glibenclamide block [2]. Since we used the common expression system to compare the taurine potency, the similarity in the extent of taurine inhibition indicates that different SUR shares a common mechanism for the taurine-mediated channel closure, which eventually occurs at Kir6.2.

Recent reports [9,11] have excluded the possibilities of taurine interaction with certain sites on nucleotide-binding domains in SUR responsible for MgADP-mediated K_{ATP}-channel activation, or with Kir6.2 responsible for ATP-mediated K_{ATP}-channel inhibition. No significant participation of Kir6.2 can be further supported by the present study showing that the glibenclamide and repaglinide sensitivities for the low-affinity binding site on Kir6.2 were not changed by taurine, whereas the high-affinity site on SUR was largely affected. The high-affinity site lies on SUR, as it is only present when SUR is coexpressed with Kir6.2, but the low-affinity site is independent of SUR, as a similar block is seen when Kir6.2 is expressed in the absence of SUR [22]. The low-affinity site is of no clinical relevance, because the concentrations required to inhibit the Kir6.2 subunit are much higher than those found in the plasma of patients treated with the drugs [20]. In the absence of added nucleotides, high-affinity inhibition of the K_{ATP} current by sulfonylurea is not complete, but reaches a maximum of 60–80% (e.g. see Fig. 3b). Though there are several possible explanations for this finding, some experimental evidences have demonstrated that the channel can still open, even when each of its four SUR subunits has bound sulfonylurea, not that some percentage of the channels are in a state in which they do not bind drug with high-affinity [20].

The high-affinity binding site for sulfonylurea drugs is believed to be made up from at least two distinct regions of SUR [18]. The residue S1237 in the cytosolic loop linking TM 15 and 16 of SUR has been implicated in binding the drugs containing the sulfonylurea moiety [27], while unidentified residues in TM 5–6 loop has been known to bind the benzamido moiety-containing drugs as determined by [^{3}H] glibenclamide-binding studies [31]. Cloned K_{ATP}-channels in response to gliclazide or tolbutamide, which have only the sulfonylurea moiety to bind SUR, fail to be sensitized by taurine. In contrast, glibenclamide or repaglinide that has the benzamido moiety made it possible in the presence of taurine. These data do not imply that only the benzamido-binding site on SUR interacts with taurine. However, it is clear that the site, probably a domain in the cytosolic loop of TM 5–6, is critical for the interaction with taurine. Further mutant-channel studies using the modified cytosolic loop remain to be determined.

Opening of K_{ATP}-channels in response to metabolic stress leads to inhibition of cellular electrical activity [32]. When muscular or neuronal cells are exposed to ischemia or hypoxia, the intracellular content of taurine decreases [33], removing its inhibition of K_{ATP}-channels. This can induce an earlier activation of K_{ATP}-channel activity to save cellular energy [7,10,11]. Since most tissues contain taurine at concentrations that exceed 10 mM [34], the inhibitory effect of taurine on the K_{ATP}-channel activity that we observed is likely to be physiologically relevant. In type 2 diabetic patients, impermeable sorbitol accumulates within cells, which results in increased taurine excretion and decreased taurine levels in blood or tissues [35]. If a subsequent hypoxic insult attacks the taurine-deficient tissues, it could exacerbate various diabetic complications, such as retinopathy, cardiomyopathy, and atherosclerosis [36]. Taken together with our results, the proper maintenance of taurine levels in a resting condition should be considered in the first place against a potential ischemic stress.

Several cytosolic agents are known to modulate the K_{ATP}-channel, including nucleotides, oleoyl CoA [37] and the membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PIP_2) [38]. PIP_2 and Mg-nucleotides (MgADP and MgATP) reduce the channel open probability [39], whereas ATP produces an apparent channel inhibition. Likewise, it seems possible that the physiological concentration of intracellular taurine may also be an important factor in setting the resting K_{ATP}-channel activity.

In conclusion, taurine inhibits pancreatic beta-cell, skeletal or cardiac muscle, and vascular smooth muscle-types of K_{ATP}-channels to a similar extent in potency when expressed in the same condition. It seems to share a common mechanism for the taurine inhibition irrespective of the types of SUR, possibly through the benzamido moiety-binding site on SUR.

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