Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 67 (2004) 1089–1096
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Inhibition of ATP-sensitive K^+ channels by taurine through a benzamido-binding site on sulfonylurea receptor 1

Eun-Jee Park[a,1], Jae Hoon Bae[b,1], So-Yeon Kim[b], Jeong-Geun Lim[a], Won-Ki Baek[a], Taeg Kyu Kwon[a], Seong-il Suh[a], Jong-Wook Park[a], In-Kyu Lee[a], Frances M. Ashcroft[c], Dae-Kyu Song[b,*]

[a] *Chronic Disease Research Center, Keimyung University School of Medicine, 194 Dongsan-Dong, Jung-Gu, Daegu 700-712, South Korea*
[b] *Department of Physiology, 194 Dongsan-Dong, Jung-Gu, Daegu 700-712, South Korea*
[c] *University Laboratory of Physiology, Parks Road, Oxford, OX1 3PT, UK*

Received 14 July 2003; accepted 3 November 2003

Abstract

ATP-sensitive potassium (K_{ATP}) channels in pancreatic β-cells comprise sulfonylurea receptor (SUR) 1 and inwardly-rectifying potassium channel (Kir) 6.2 subunits. We have evaluated the effect of intracellular taurine on K_{ATP} channel activity in rat pancreatic β-cells using the patch-clamp technique. The mechanism of taurine action was also examined using recombinant K_{ATP} channels. The islets and single β-cells from male Sprague–Dawley rats were collected by collagenase digestion technique. Single K_{ATP} channel currents were recorded by the inside–out mode at a membrane potential of −60 mV. Cytosolic free-Ca^{2+} concentration ($[Ca^{2+}]_c$) and insulin secretory capacity were measured by the dual-excitation fluorimetry and radioimmunoassay, respectively. The native β-cell K_{ATP} channel was directly inhibited by taurine in a dose-dependent manner. Taurine did not influence ATP-mediated inhibition or MgADP-induced activation of the channel activity. The sensitivity of the K_{ATP} channel to glybenclamide, but not gliclazide, was enhanced by taurine. Glybenclamide elicited a greater increase in $[Ca^{2+}]_c$ and increased insulin secretion in the β-cells when pretreated with taurine. Taurine did not inhibit Kir6.2ΔC36 currents, a truncated form of Kir6.2, expressed in *Xenopus* oocytes without SUR. These results demonstrate that taurine inhibits the K_{ATP} channel activity in the β-cells, interacting with a benzamido-binding site on SUR1, but not Kir6.2.

Keywords: Taurine; ATP-sensitive potassium channel; Sulfonylurea; Ca^{2+} concentration; Insulin; Pancreatic β-cell

1. Introduction

Taurine (2-amino ethanesulfonic acid), is a low-molecular weight organic cellular constituent that is found in high concentrations in almost all mammalian tissues. As humans biosynthesize only small amounts of taurine, dietary supplements of taurine are needed [1]. The ability of taurine to treat cardiovascular [2,3], skeletal muscle [4], and neuronal diseases [5] that are mainly caused by ischaemia or hypoxia has been extensively studied. In contrast to carboxylic amino acids, taurine is a sulfur-containing, zwitterionic β-amino acid at normal physiological pH, with a high hydrophilicity. Its lack of lipophilicity thus aids the ability of taurine to act as an important organic osmolyte. In addition, due to its polyvalence, it readily interacts with neutral phospholipids [6] and thereby modifies the functions of membrane proteins such as ion channels [7,8] and other plasmalemmal proteins [9,10], as well as proteins in the membranes of intracellular organelles [11].

Taurine inhibits K_{ATP} channels in cardiac tissues [12,13] and skeletal muscles [14]. However, the exact mechanism of taurine action is still unknown. The effect of taurine on the β-cell K_{ATP} channel has also not been examined. The K_{ATP} channel is composed of two subunits: SUR and Kir. β-Cells possess the Kir6.2/SUR1 K_{ATP} channel [15,16] while the cardiac and skeletal K_{ATP} channels are composed

* Corresponding author. Tel.: +82-53-250-7490; fax: +82-53-252-1605.
E-mail address: dksong@kmu.ac.kr (D.-K. Song).
[1] Author contributed equally.

Abbreviations: P_o, open probability; IC_{50}, half-maximal concentration for inhibition; *h*, Hill coefficient; K_{ATP} channel, ATP-sensitive potassium channel; $[Ca^{2+}]_c$, cytosolic free-Ca^{2+} concentration; SUR, sulfonylurea receptor; Kir, inwardly-rectifying potassium channel; ATP, adenosine triphosphate; ADP, adenosine diphosphate; N, number of data.

0006-2952/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2003.11.003

of Kir6.2/SUR2A [17,18]. Smooth muscle K_{ATP} channels consist of Kir6.1/SUR2B or Kir6.2/SUR2B [19,20]. The Kir subunit forms a pore while the SUR subunit is a regulatory subunit that endows the Kir with sensitivity to drugs such as the inhibitory sulfonylureas [21] (e.g. glybenclamide) and the stimulatory K^+ channel openers [22].

We have assessed the effect of intracellular taurine on properties of β-cell K_{ATP} channels.

2. Materials and methods

2.1. Preparation of islets and single β-cells from rat pancreas

Islets of Langerhans were isolated from the pancreas of male Sprague–Dawley rat weighing 200–250 g by a collagenase digestion technique [23]. Briefly, the animals were first anaesthetized by Nembutal. Collagenase (Type V; Sigma) dissolved at 1 mg/mL in Hank's Balanced Salts solution (HBSS) was transfused via the common bile duct into the pancreatic ducts. The dissected pancreas was transferred into a 15-mL capped conical tube for a 15-min incubation at 37° in a shaking water bath. Islets were picked out with a 10-μL pipette on a stereoscope (15×) and placed into Krebs Ringer Bicarbonate Buffer (KRBB) solution containing 10% bovine serum albumin (BSA), penicillin (100 unit/mL), and streptomycin (0.1 mg/mL). Islets were dissociated into single cells by trituration using a fire-polished Pasteur pipette, and a 1-mL syringe employing increasingly smaller needle diameters (20–26 gauge).

For patch-clamp experiment, single cells were dispersed onto cover glasses (8 mm × 3 mm) in a 35-mm culture dish and incubated in RPMI-1640 media with 11.1 mM glucose, 10% fetal bovine serum (FBS), and antibiotics in a humidified incubator at 37° with 5% CO_2 and balanced air. The islets used to measure insulin secretion and the single cells used for $[Ca^{2+}]_c$ measurements were incubated in the same RPMI media without FBS. After 24-hr incubation, cells were exposed to 0.3 or 3 mM taurine (taurine groups), and then incubated at 37° for ≥24 hr before experiments. During experiments, taurine was present in all solutions for the taurine groups.

2.2. Molecular biology and oocyte collection for cloned K_{ATP} channels

Mouse Kir6.2 (Genbank D50581 [16]) cDNA was cloned in the pBF vector. To obtain currents with Kir6.2 alone independent of SUR1, a truncated form of Kir6.2 (Kir6.2ΔC36) in which the last 36 amino acids of the C terminus had been deleted was made by introduction of a stop codon at the appropriate residues by site-directed mutagenesis [24]. Capped mRNA was prepared using the mMESSAGE mMACHINE large scale *in vitro* transcription kit (Ambion), as previously described [25].

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 at room temperature for 60 min with 1.0 mg/mL collagenase (Sigma, type V) and manually defolliculated. Oocytes were injected with ∼0.1 ng of Kir6.2ΔC36 mRNA. The final injection volume was 50 nL per oocyte. Isolated oocytes were maintained in Barth's solution at 19° and studied 1–4 days after injection [25].

2.3. Electrophysiological measurement of K_{ATP} channel activity

The inside–out configuration of the conventional patch-clamp technique was used for the single channel recording of K_{ATP} channel activity. Patch pipettes were pulled from borosilicate thin-walled (for β-cells) or thick-walled (for oocytes) glass capillaries (World Precision), fire-polished, and coated with Sylgard resin (Dow Corning) near the tip. The pipette had resistance between 3 and 5 MΩ (β-cells) or 250–500 kΩ (oocytes). The cells were mounted on an inverted microscope (Axiovert 135; Carl Zeiss) and perfused with the bath solution indicated below. The single channel currents were recorded using an Axopatch 200A (β-cells) or Geneclamp-500 (oocytes) patch-clamp amplifier (Axon), passed onto an A/D converter (Digidata 1200A; Axon), stored on videotape via a pulse code modulator (VR-10B; Instrutec), and later analyzed with Pclamp 8.2 software (Axon) on a computer. The experiments were performed at room temperature.

2.3.1. Native β-cell K_{ATP} channel recording

Data were filtered at 1 kHz and sampled at 5 kHz with a membrane potential clamped at −60 mV. The extent of channel activity was expressed as P_o. The relative channel activity in the presence of a test chemical was described as P_o/P_{oc}, where P_{oc} is the P_o taken as the mean of that obtained before and after application of the test compound except for glybenclamide, of which effect is irreversible during experiment for the β-cell type channel. Each P_o was calculated with data points during the last 30 s interval of the test solution. The P_o/P_{oc} was then fitted into the Hill equation by using Origin 5.0 software (MicroCal).

$$\frac{P_o}{P_{oc}} = \left[1 + \left(\frac{[A]}{IC_{50}}\right)^h\right]^{-1}$$

where [A] is the concentration of a chemical, IC_{50} is the half-maximal concentration for inhibition of the chemical, and h is the Hill coefficient.

In the inside–out experiment, cells were bathed in a solution (intracellular) composed of (in mM) 107 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, 10 EGTA, and pH 7.2 with KOH. The pipette (external) solution contained (in mM) 140 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, 10 HEPES, and pH 7.4 with KOH.

2.3.2. Cloned K_{ATP} channel recording

Macroscopic currents were recorded from giant excised inside–out patches at a holding potential of 0 mV [25]. Currents were evoked by repetitive 3 s voltage ramps from −110 to +100 mV. Currents were filtered at 10 kHz, digitized at 0.4 kHz and resampled at 20 Hz for presentation in the figures. The pipette and bath solution were the same as those for the native β-cells.

2.4. Measurement of the change in $[Ca^{2+}]_c$

To observe the effect of taurine pretreatment on the glybenclamide-induced $[Ca^{2+}]_c$ changes in the β-cells, the cultured β-cells were mounted onto a cover slip (25 mm diameter) and bathed in a pH 7.4 physiological solution containing (mM) 126 NaCl, 5 KCl, 1 $CaCl_2$, 1.2 $MgCl_2$, 10 HEPES with pH 7.4.

The calcium indicator dye Fura-2 acetoxymethyl ester (Fura-2/AM) (3 μM) was then added to the cells for 30 min for the micro-fluorescent imaging of $[Ca^{2+}]_c$. This incubation was followed by a 30-min wash in dye-free physiological solution to allow esterase conversion into free Fura-2. A cover slip was placed on the stage of an inverted microscope and fluorescence was measured by an InCa dual-wavelength system (Intracellular Imaging), with excitation and emission wavelengths set at 340/380 and 510 nm, respectively. Ratio images were processed every 5 s and converted into $[Ca^{2+}]_c$ relative to a range of values obtained by measuring Fura-2 in the presence of a known concentration of calcium (Calcium Calibration Buffer Kit; Molecular Probe).

2.5. Measurement of insulin secretory capacity for islets

Insulin secretory capacity was measured by the batch incubation method as previously reported [26]. Normal taurine concentration in the plasma is about 0.3 mM [27,28], but we tested taurine at both 0.3 and 3 mM, because 3 mM taurine was reported to induce more remarkable results in some experiments [28]. Taken from the RPMI-media, the islets were then incubated for 1 hr at 37° in a modified KRBB solution containing (mM) 114 NaCl, 4.4 KCl, 1.28 $CaCl_2$, 1 $MgSO_4$, 29.5 $NaHCO_3$, 10 HEPES, 5 glucose, 0.1% BSA, and pH 7.4 adjusted with NaOH. Ten islets [28] each was then placed into each one of batches of 48-well plate containing the KRBB solution plus varying glybenclamide concentrations. The islets were cultured for 1 hr at 37° and then an aliquot was taken from each well and centrifuged (700 *g*, 5 min). The supernatant (200 μL) was carefully collected and stored in a freezer (−20°) for later radioimmunoassays. Rat insulin assay kits were purchased from Linco Research Inc.

2.6. Materials

Fura-2/AM was obtained from Molecular Probes and dissolved in dimethyl sulfoxide (DMSO). Taurine was purchased from Tocris. BSA and FBS were purchased from Gibco Invitrogen and Hyclone, respectively. All other chemicals were obtained from Sigma Chemical Co.

Drug-containing bath solutions were made just before the experiment. ATP (10 mM) and ADP (10 mM) in the bath solution, gliclazide (50 mM) and glybenclamide (10 mM) in DMSO were prepared as stock solutions. They were diluted immediately before use to the final concentrations indicated. Flow rate was adjusted to make a complete change of the bath solution within 10 s.

2.7. Statistical analysis

Data were expressed as means ± SEM. Statistical significance was evaluated by unpaired Student's *t* test when only two groups were involved, and by ANOVA followed by a test of Dunnett when two experimental groups are

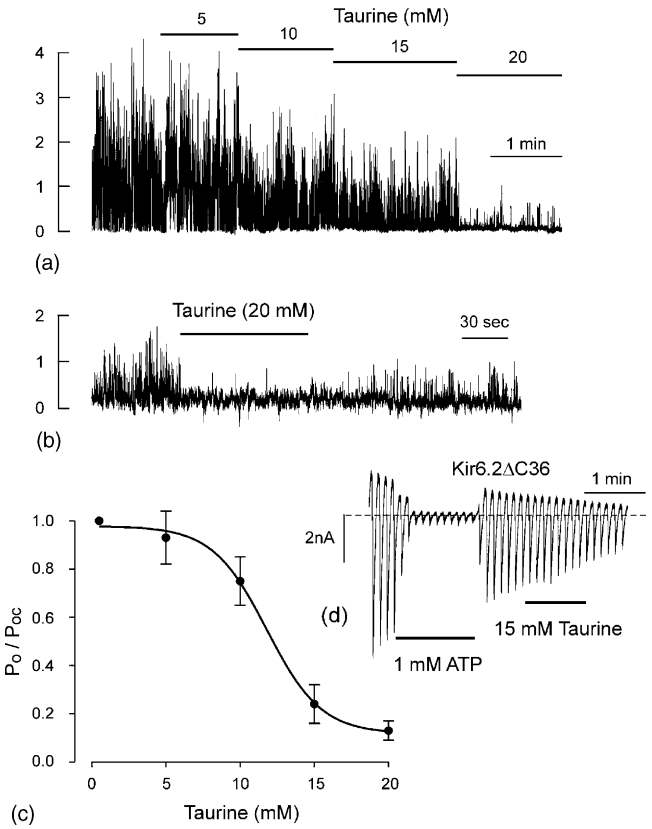

Fig. 1. Inhibition of K_{ATP} channel currents by taurine. (a) A representative trace of K_{ATP} channel activity in a native β-cell showing a dose-dependent inhibition in response to taurine. Inside–out mode at a membrane potential of −60 mV. Taurine was applied to the intracellular side. The vertical scale indicates the number of channels. (b) A trace showing a reversible inhibition by taurine of K_{ATP} channel current in a native β-cell. (c) Dose–response relationship of K_{ATP} channel activity to taurine. P_o in the presence of taurine was normalized relative to the mean of P_o obtained before and after exposure to taurine (P_{oc}). The symbols represent the mean, and vertical bars indicate SEM. Data were fitted into the Hill equation, yielding an IC_{50} of 12.3 mM and *h* of 4.8 (N = 6). (d) A macroscopic cloned Kir6.2ΔC36 current recorded in response to a series of voltage ramps from −110 to +100 mV. Inside–out mode from the oocyte membrane at a holding potential of 0 mV. Taurine (15 mM) and ATP (1 mM) were applied during the period indicated by the bars.

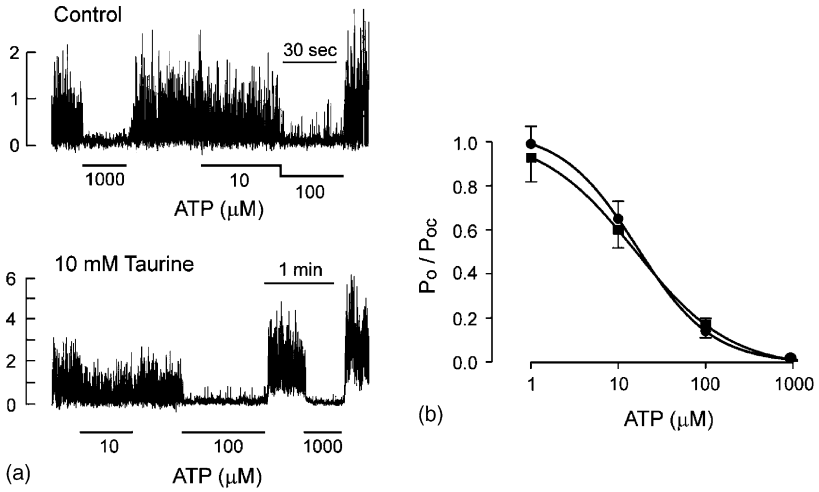

Fig. 2. Effect of taurine on ATP-induced inhibition of K_{ATP} channel currents in native β-cells. (a) Traces of K_{ATP} channel activity showing ATP-induced current inhibition in the absence (upper) or presence (lower) of 10 mM taurine. Inside–out mode at a membrane potential of −60 mV. Taurine and ATP were applied to the intracellular side. The vertical scales indicate the number of channels. (b) Dose–response relationships of K_{ATP} channel activity to ATP in the presence (■) or absence (●) of 10 mM taurine. P_o in the presence of ATP was normalized relative to the mean of P_o obtained before and after exposure to ATP (P_{oc}). The symbols represent the mean, and vertical bars indicate SEM. Data were fitted into the Hill equation, yielding an IC_{50} of 16.4 μM and h of 1 (N = 4) in the absence of taurine, and an IC_{50} of 15.8 μM and h of 0.8 (N = 6) in the presence of taurine.

compared with a control group; $P < 0.05$ was considered significant.

3. Results

3.1. Direct effect of taurine on K_{ATP} channel activity through SUR1, not Kir6.2

When applied to the intracellular side of the β-cell membrane, taurine inhibited the K_{ATP} channel activity in a dose-dependent and reversible manner (Fig. 1a and b). It was fitted into the Hill equation, showing an IC_{50} of 12.3 ± 0.4 mM (N = 6) and h of 4.8 (Fig. 1c). However, Kir6.2ΔC36 current recorded in the oocyte membrane was not inhibited by taurine, suggesting that taurine acts on SUR1, not Kir6.2 (Fig. 1d).

3.2. Effect of taurine on ATP-mediated inhibition and MgADP-induced activation of β-cell K_{ATP} channel activity

Taurine was tested at a concentration near the IC_{50} (10 mM) on the nucleotide sensitivity of the K_{ATP} channel. ATP inhibited the channel current to a similar extent in the absence and presence of taurine, the IC_{50} being

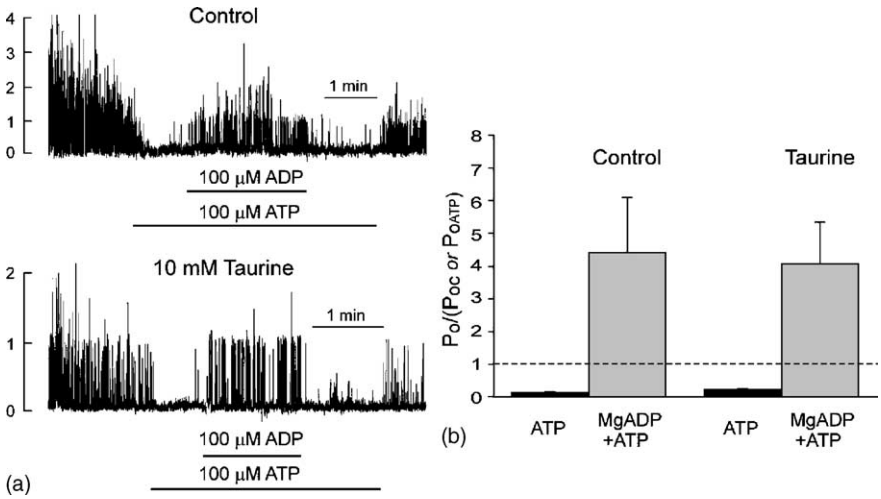

Fig. 3. Effect of taurine on MgADP-induced activation of K_{ATP} channel currents in the presence of 100 μM ATP. (a) Traces of K_{ATP} channel activity in native β-cells showing the activation by MgADP in the absence (upper) or presence (lower) of 10 mM taurine. Inside–out mode at a membrane potential of −60 mV. Taurine, MgADP and ATP were applied to the intracellular side. The vertical scales indicate the number of channels. (b) Mean P_o of K_{ATP} channel activity in response to 100 μM ATP (■) or ATP plus 100 μM MgADP (▨) with (right, N = 7) or without (left, N = 5) 10 mM taurine. P_o in the presence of MgADP was normalized relative to the P_o measured in the presence of ATP only. The symbols represent the mean and SEM.

16.4 ± 0.3 μM (N = 4) and 15.8 ± 0.4 μM (N = 6); *h* of 1 and 0.8, respectively (Fig. 2a and b). MgADP (100 μM) in the presence of 100 μM ATP increased channel activity (Fig. 3a). The difference in the magnitude of MgADP activation was not significant between taurine and taurine-free groups (4.1 ± 1.3 μM, N = 7; 4.4 ± 1.7 μM, N = 5, respectively; Fig. 3b). These observations suggest that neither ATP inhibition nor MgADP activation of the channel activity are modulated by intracellular taurine.

3.3. Effect of taurine on sulfonylureas-mediated inhibition of β-cell K_{ATP} channel activity

We next evaluated the interaction of taurine with the sulfonylureas, glybenclamide and gliclazide. Glybenclamide interacts with two high-affinity binding regions of the drug-binding pocket on SUR1 (sulfonylurea- and benzamido-binding sites) [29] whereas gliclazide only interacts with one (the sulfonylurea-binding site) [30,31]. In the presence of taurine (10 mM), the sensitivity of β-cell K_{ATP} channel to glybenclamide was increased (Fig. 4a and b). Figure 4c shows that the glybenclamide concentration–inhibition curve was markedly left-shifted by taurine, and the IC_{50} decreased from 1.7 ± 0.8 nM (N = 8) in the absence to 0.1 ± 0.1 nM (N = 6) in the presence of taurine. The Hill coefficient was unchanged: $h = 0.5$ and 0.4, respectively. However, taurine (10 mM) failed to enhance the sensitivity of the K_{ATP} channel to gliclazide (Fig. 5a and b), yielding an IC_{50} of 30 ± 0.001 nM and *h* of 1.9 (N = 4) in the absence of taurine, and an IC_{50} of 30 ± 0.004 nM and *h* of 1.2 (N = 4) in the presence of taurine (Fig. 5c). It suggests that taurine does not interact with the sulfonylurea-binding site for glybenclamide.

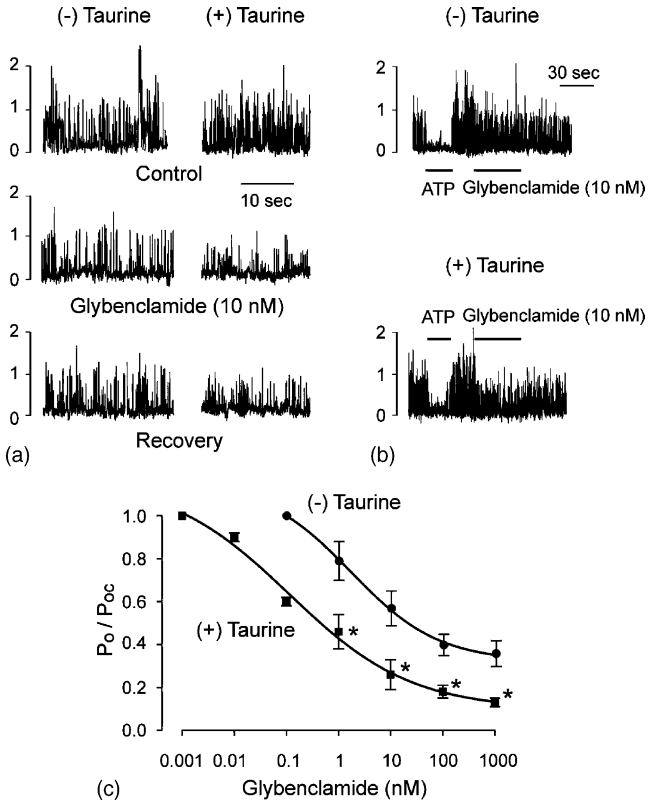

Fig. 4. Effect of taurine on glybenclamide-mediated inhibition of K_{ATP} channel currents in native β-cells. (a, b) Traces of K_{ATP} channel activity showing the glybenclamide-induced inhibition in the absence or presence of 10 mM taurine. Inside–out mode at a membrane potential of −60 mV. Taurine and glybenclamide were applied to the intracellular side. The vertical scales indicate the number of channels. (c) Glybenclamide concentration–inhibition curve of K_{ATP} channel activity in the presence (■) or absence (●) of taurine. P_o measured in the presence of glybenclamide was normalized relative to the P_o measured immediately before exposure to glybenclamide (P_{oc}). The symbols represent the mean, and vertical bars indicate SEM. Data were fitted into the Hill equation, yielding an IC_{50} of 1.7 nM and *h* of 0.5 (N = 8) in the absence of taurine, and an IC_{50} of 0.1 nM and *h* of 0.4 (N = 6) in the presence of taurine. $^*P < 0.05$ vs. the value of (−)taurine at the same concentration of glybenclamide using unpaired *t* test.

3.4. Effect of taurine on glybenclamide-induced change in $[Ca^{2+}]_c$ and insulin release

The closure of K_{ATP} channels by sulfonylureas should increase $[Ca^{2+}]_c$ in the β-cell and thereby induce insulin

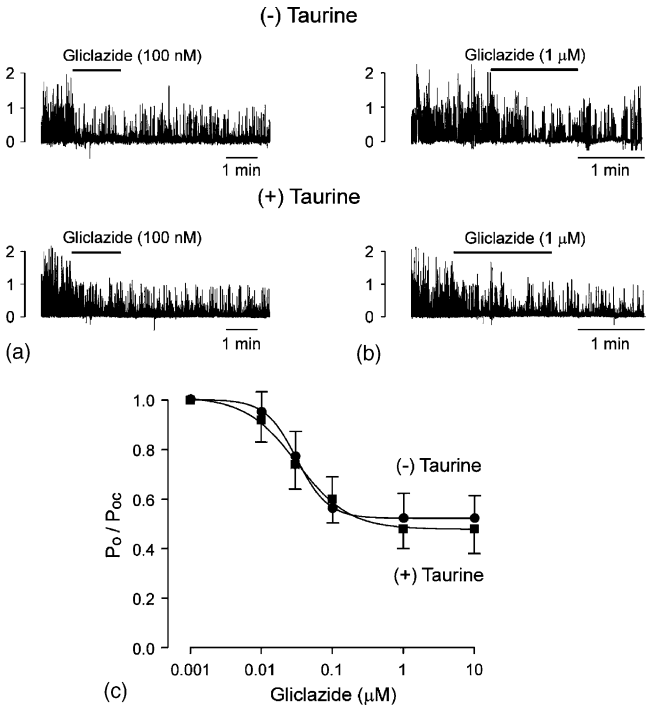

Fig. 5. Effect of taurine on gliclazide-mediated inhibition of K_{ATP} channel currents in native β-cells. (a, b) Traces of K_{ATP} channel activity showing the gliclazide-induced inhibition in the absence or presence of 10 mM taurine. Inside–out mode at a membrane potential of −60 mV. Taurine and gliclazide were applied to the intracellular side. The vertical scales indicate the number of channels. (c) Gliclazide concentration–inhibition curve of K_{ATP} channel activity in the presence (■) or absence (●) of taurine. P_o in the presence of gliclazide was normalized relative to the mean of P_o obtained before and after exposure to gliclazide (P_{oc}). The symbols represent the mean, and vertical bars indicate SEM. Data were fitted into the Hill equation, yielding an IC_{50} of 30 nM and *h* of 1.9 (N = 4) in the absence of taurine, and an IC_{50} of 30 nM and *h* of 1.2 (N = 4) in the presence of taurine.

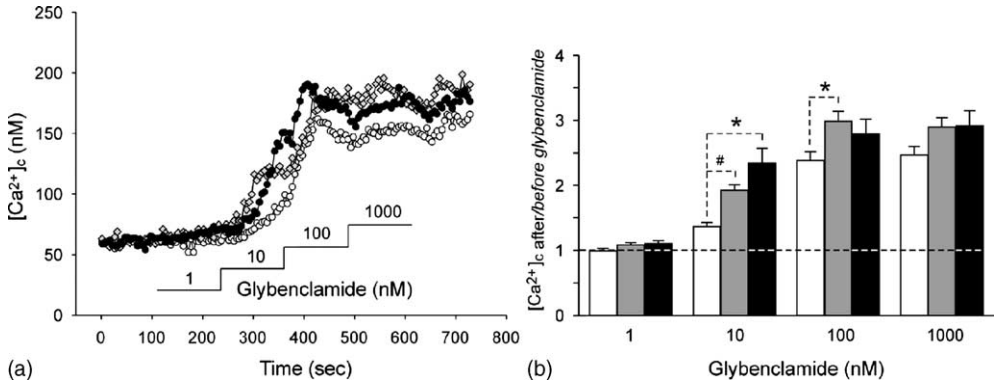

Fig. 6. Effect of taurine pretreatment on the glybenclamide-induced increase in $[Ca^{2+}]_c$. (a) The $[Ca^{2+}]_c$ levels induced by a serial application of glybenclamide without (○, N = 22) or with taurine pretreatment (◇: 0.3 mM, N = 15; ●: 3 mM, N = 19). Each experimental data point represents the mean calculated from at least 20 to 30 individually measured cells from three separate cultures. (b) Relative response of $[Ca^{2+}]_c$ to various doses of glybenclamide without (□) or with taurine pretreatment (▩: 0.3 mM; ■: 3 mM). $[Ca^{2+}]_c$ measured in the presence of glybenclamide was normalized with that measured before glybenclamide exposure at each group. Data were collected during the last 30 s at each period. The symbols represent the normalized mean, and vertical bars indicate SEM. The dashed line indicates the level of $[Ca^{2+}]_c$ before glybenclamide application. $^{\#}P < 0.05$; $^{*}P < 0.05$ vs. the value of no taurine group at the same concentration of glybenclamide using analysis of variance followed by test of Dunnett.

secretion. In control cells, addition of glybenclamide to the extracellular solution triggered an increase in $[Ca^{2+}]_c$, in a manner that was dependent on the glybenclamide concentration (Fig. 6a and b). In cells pretreated with 0.3 or 3 mM taurine, the $[Ca^{2+}]_c$ increase induced by glybenclamide was greater than that of control cells. Glybenclamide-stimulated insulin secretion of islet cells was also higher in taurine-pretreated cells than in control cells (Fig. 7). Thus, the ability of taurine to enhance the inhibitory action of glybenclamide on the K_{ATP} channel is physiologically relevant, as this effect is associated with a greater $[Ca^{2+}]_c$, and elevated insulin secretion by the β-cells.

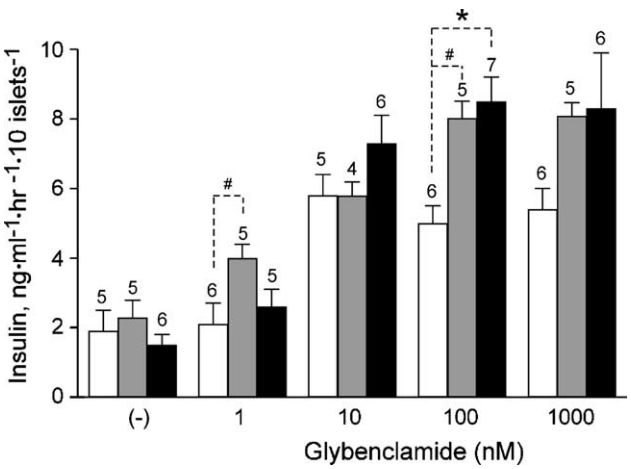

Fig. 7. Insulin secretory capacity of islets in response to glybenclamide without (□) or with taurine pretreatment (▩: 0.3 mM; ■: 3 mM). The mean of data from three consecutive batches with the same condition was taken as one data point. The number of data points is given above each bar of the mean and SEM, obtained in at least three separate experiments. $^{\#}P < 0.05$; $^{*}P < 0.05$ vs. the value of no taurine group at the same concentration of glybenclamide using ANOVA followed by test of Dunnett.

4. Discussion

In this study, we show that taurine causes a dose-dependent inhibition of K_{ATP} channel, when measured in inside–out patches excised from rat pancreatic β-cells. This finding is consistent with previously published observations that taurine inhibits the activity of the K_{ATP} channels found in skeletal [14] and cardiac [12,13] muscle. The IC_{50} for the taurine-mediated block of the β-cell K_{ATP} current was 12.3 mM, close to that measured in cardiac myocytes (13.5 mM [12]), suggesting that the different type of SUR subunits is not critical for the taurine action. Since most tissues contain taurine at concentrations that exceed 10 mM [32], the inhibitory effect of taurine on the K_{ATP} channel activity that we observed is likely to be physiologically relevant. We found that taurine does not modulate the K_{ATP} channel sensitivity to ATP and MgADP. Furthermore, taurine could not inhibit the Kir6.2ΔC36 current. It is known that the principal site at which ATP acts to mediate K_{ATP} channel inhibition resides on Kir6.2 and that SUR1 further enhances the ATP sensitivity of Kir6.2 [24]. By contrast, the activatory action of MgADP is mediated via nucleotide-binding domain on SUR1 [33]. Taken together, it may be that the functional modifications of the K_{ATP} channels induced by taurine do not involve the nucleotide-binding domain of SUR1 or the Kir6.2. But, it cannot be excluded that taurine interaction with either Kir6.2 or SUR1 may require the terminal 36 amino acids that are deleted from the mutant Kir isoform.

The inhibitory effect of glybenclamide on native β-cell K_{ATP} channel was potentiated in the presence of taurine. Recent studies have revealed that SUR1 has two binding sites for glybenclamide: a sulfonylurea-binding site and a benzamido-binding site. These loops lie in close proximity in the three-dimensional structure of the K_{ATP} channel [29]. In contrast, SUR2A in cardiac and skeletal muscles

has only the benzamido-binding site [34]. The β-cell specific sulfonylureas that bear only sulfonylurea moiety in molecules, such as gliclazide, tolbutamide, etc. may be bound to only the former binding site. It has been reported that the K_{ATP} channels (Kir6.2/SUR2A) in skeletal muscles were more sensitized to glybenclamide in the presence of taurine, suggesting taurine can interfere with the benzamido-binding site. We tested gliclazide, which is proposed to act only the sulfonylurea-binding site on SUR1 [35], and observed that the gliclazide sensitivity of K_{ATP} channels would not be enhanced by taurine in contrast to that of glybenclamide. These findings suggest that taurine may not interact with the sulfonylurea-binding site on SUR1, but interacts with the benzamido-binding site.

Taurine affects many different ion channels in cell membranes including the Na^+, Ca^{2+}, Cl^-, and K^+ channels, but it is difficult to identify taurine-binding sites on these ion channels. Indeed, high-affinity taurine-binding sites have only been identified on Na^+-dependent and -independent taurine transporters that mediate taurine influx [36] and efflux [37], respectively. Low-affinity taurine binding is reported for the neutral membrane phospholipids [6], and may alter membrane architecture and fluidity. In skeletal muscle fibers, the inhibitory effect of taurine on K_{ATP} channel activity is independent of the functional state of the channel [14]; supporting the notion that taurine may allosterically modify the K_{ATP} channel activity by binding to the polar phase of the membrane phospholipids that are functionally related to the SUR.

In conclusion, the β-cell-type K_{ATP} channel (Kir6.2/SUR1) is directly inhibited by intracellular taurine. The mechanism of taurine action may be mediated through the benzamido-binding site on SUR1, but not Kir6.2.

Acknowledgments

This work was supported by grant no. R13-2002-028-01003-0, R05-2002-000-00558-0 from the Basic Research Program of the KOSEF.

References

[1] Huxtable RJ. Physiological actions of taurine. Physiol Rev 1992;72:101–63.
[2] Satoh H. Cardiac actions of taurine as a modulator of the ion channels. Adv Exp Med Biol 1998;442:121–8.
[3] Petty MA, Kintz J, DiFrancesco GF. The effects of taurine on atherosclerosis development in cholesterol-fed rabbits. Eur J Pharmacol 1990;180:119–27.
[4] Pierno S, De Luca A, Camerino C, Huxtable RJ, Camerino DC. Chronic administration of taurine to aged rats improves the electrical and contractile properties of skeletal muscle fibers. J Pharmacol Exp Ther 1998;286:1183–90.
[5] Huxtable RJ. Taurine in the central nervous system and the mammalian actions of taurine. Prog Neurobiol 1989;32:471–533.
[6] Huxtable RJ, Sebring LA. Towards a unifying theory for the actions of taurine. Trends Pharmacol Sci 1986;7:481–5.
[7] De Luca A, Pierno S, Camerino DC. Effect of taurine depletion on excitation-contraction coupling and Cl^- conductance of rat skeletal muscle. Eur J Pharmacol 1996;296:215–22.
[8] Steele DS, Smith GL, Miller DJ. The effects of taurine on Ca^{2+} uptake by the sarcoplasmic reticulum and Ca^{2+} sensitivity of chemically skinned rat heart. J Physiol 1990;422:499–511.
[9] Igisu H, Izumi K, Goto I, Kina K. Effects of taurine on the ATPase activity in the human erythrocyte membrane. Pharmacology 1976;14:362–6.
[10] Sebring LA, Huxtable RJ. Taurine modulation of calcium binding to cardiac sarcolemma. J Pharmacol Exp Ther 1985;232:445–51.
[11] Lombardini JB. Effects of taurine and mitochondrial metabolic inhibitors on ATP-dependent Ca^{2+} uptake in synaptosomal and mitochondrial subcellular fractions of rat retina. J Neurochem 1988;51:200–5.
[12] Han J, Kim E, Ho WK, Earm YE. Blockade of the ATP-sensitive potassium channel by taurine in rabbit ventricular myocytes. J Mol Cell Cardiol 1996;28:2043–50.
[13] Satoh H. Direct inhibition by taurine of the ATP-sensitive K^+ channel in guinea pig ventricular cardiomyocytes. Gen Pharmacol 1996;27:625–7.
[14] Tricarico D, Barbieri M, Camerino DC. Taurine blocks ATP-sensitive potassium channels of rat skeletal muscle fibres interfering with the sulphonylurea receptor. Br J Pharmacol 2000;130:827–34.
[15] Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement JP, Boyd III AE, Gonzalez G, Herrera-Sosa H, Nguy K, Bryan J, Nelson DA. Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. Science 1995;268:423–6.
[16] Inagaki N, Gonoi T, Clement JP, Namba N, Inazawa J, Gonzalez G, Aguilar-Bryan L, Seino S, Bryan J. Reconstitution of IK_{ATP}: an inward rectifier subunit plus the sulfonylurea receptor. Science 1995;270:1166–70.
[17] Inagaki N, Gonoi T, Clement JP, Wang CZ, Aguilar-Bryan L, Bryan J, Seino S. A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels. Neuron 1996;16:1011–7.
[18] Chutkow WA, Simon MC, Le Beau MM, Burant CF. Cloning, tissue expression, and chromosomal localization of SUR2, the putative drug-binding subunit of cardiac, skeletal muscle, and vascular K_{ATP} channels. Diabetes 1996;45:1439–45.
[19] Isomoto S, Kondo C, Yamada M, Matsumoto S, Higashiguchi O, Horio Y, Matsuzawa Y, Kurachi Y. A novel sulfonylurea receptor forms with BIR (Kir6.2) a smooth muscle type ATP-sensitive K^+ channel. J Biol Chem 1996;271:24321–4.
[20] Yamada M, Isomoto S, Matsumoto S, Kondo C, Shindo T, Horio Y, Kurachi Y. Sulphonylurea receptor 2B and Kir6.1 form a sulphonylurea-sensitive but ATP-insensitive K^+ channel. J Physiol 1997;499:715–20.
[21] Proks P, Reimann F, Green N, Gribble FM, Ashcroft FM. Sulfonylurea stimulation of insulin secretion. Diabetes 2002;51:S368–76.
[22] Garrino MG, Plant TD, Henquin JC. Effects of putative activators of K^+ channels in mouse pancreatic beta-cells. Br J Pharmacol 1989;98:957–65.
[23] Findlay I, Dunne MJ. ATP maintains ATP-inhibited K^+ channels in an operational state. Pflügers Arch 1986;407:238–40.
[24] Tucker SJ, Gribble FM, Zhao C, Trapp S, Ashcroft FM. Truncation of Kir6.2 produces ATP-sensitive K^+ channels in the absence of the sulphonylurea receptor. Nature 1997;387:179–83.
[25] Gribble FM, Ashfield R, Ammälä C, Ashcroft FM. Properties of cloned ATP-sensitive K^+ currents expressed in *Xenopus* oocytes. J Physiol 1997;498:87–98.
[26] Song DK, Ahn YH, Bae JH, Park WK, Hong YS, Ho WK, Earm YE. Evidence of enhancement of malate-aspartate shuttle activity in beta cells of streptozotocin-induced non-insulin-dependent diabetic rats. Metabolism 2000;49:92–6.
[27] Reusens B, Dahri S, Snoeck A, Bennis Taleb N, Remacle C, Hoet JJ. Long-term consequences of diabetes and its complications may have a

fetal origin: experimental and epidemiological evidence. In: Cowett RM, editor. Diabetes nestle nutrition workshop series. New York: Raven Press; 1995. p. 187–98.

[28] Cherif H, Reusens B, Ahn MT, Hoet JJ, Remacle C. Effects of taurine on the insulin secretion of rat fetal islets from dams fed a low-protein diet. J Endocrinol 1998;159:341–8.

[29] Mikhailov MV, Mikhailova EA, Ashcroft SJ. Molecular structure of the glybenclamide binding site of the beta-cell K_{ATP} channel. FEBS Lett 2001;499:154–60.

[30] Gribble FM, Tucker SJ, Seino S, Ashcroft FM. Tissue specificity of sulfonylureas: studies on cloned cardiac and beta-cell K_{ATP} channels. Diabetes 1998;47:1412–8.

[31] Gribble FM, Ashcroft FM. Differential sensitivity of beta-cell and extrapancreatic K_{ATP} channels to gliclazide. Diabetologia 1999;42:845–8.

[32] Briel G, Gylfe E, Hellman B, Neuhoff V. Microdetermination of free amino acids in pancreatic islets isolated from obese-hyperglycemic mice. Acta Physiol Scand 1972;84:247–53.

[33] Gribble FM, Tucker SJ, Ashcroft FM. The essential role of the Walker A motifs of SUR1 in K-ATP channel activation by Mg-ADP and diazoxide. EMBO J 1997;16:1145–52.

[34] Gribble FM, Reimann F. Differential selectivity of insulin secretagogues. Mechanisms, clinical implications, and drug interactions. J Diabetes Complications 2003;17:S11–5.

[35] Lawrence CL, Proks P, Rodrigo GC, Jones P, Hayabuchi Y, Standen NB, Ashcroft FM. Gliclazide produces high-affinity block of K_{ATP} channels in mouse isolated pancreatic beta cells but not rat heart or arterial smooth muscle cells. Diabetologia 2001;44:1019–25.

[36] Atlas M, Bahl JJ, Roeske W, Bressler R. *In vitro* osmoregulation of taurine in fetal mouse hearts. J Mol Cell Cardiol 1984;16:311–20.

[37] Fugelli K, Thoroed SM. Taurine transport associated with cell volume regulation in flounder erythrocytes under anisosmotic conditions. J Physiol 1986;374:245–61.