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Taurine blocks ATP-sensitive potassium channels of rat skeletal muscle fibres interfering with the sulphonylurea receptor

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1 Taurine is a sulphonic aminoacid present in high amounts in various tissues including cardiac and skeletal muscles showing different properties such as antioxidative, antimyotonic and anti-schaemic effects. The cellular mechanism of action of taurine is under investigation and appears to involve the interaction of the sulphonic aminoacid with several ion channels.

2 Using the patch-clamp technique we studied the effects of taurine in rat skeletal muscle fibres on ATP-sensitive K^+ channel (K_{ATP}) immediately after excision and on channels that underwent rundown.

3 The cytoplasmic application of 20 mM of taurine reduced the K_{ATP} current; this effect was reverted by washout of the drug solution. In this experimental condition the IC_{50} was 20.1 mM. After rundown, taurine inhibited the K_{ATP} current with similar efficacy. Competition experiments showed that taurine shifted the dose-response inhibition curve of glybenclamide to the left on the log-dose axis without significantly affecting those of ATP or Ca^{2+} ion.

4 Single channel recording revealed that taurine affects the close state of the channel prolonging it and reducing the bursts duration.

5 Our data indicate that taurine inhibits the muscular K_{ATP} channel interfering with the glybenclamide site on the sulphonylurea receptor of the channel or on the site allosterically coupled to it. During ischaemia and hypoxia, the skeletal and heart muscles undergo several changes; for example, the activation of K_{ATP} channels and loss of the intracellular taurine content. The depletion of taurine during ischaemia would contribute to the early activation of K_{ATP} channels and salvage the intracellular ATP content.

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Abbreviations: APD, action potential duration; EGTA, ethylene glycol-bis(b-aminoethyl ether)-N,N,N′,N′-tetra-acetic acid; FDB, flexor digitorum brevis; GCI, chloride conductance; IC_{50}, concentration of drug needed to reduce the current by 50%; I control, current in the absence of drugs; I drug, current in the presence of drugs; K_{ATP}, ATP-sensitive K^+ channels; KCO, K^+ channel agonists; Kir, inward rectifier K^+ channel; MOPS, 3-(N-morpholino)propanesulphonic acid; *n*, Hill coefficient of the curves; Na_2ATP, adenosine-triphosphate sodium salt; P_{open}, open probability; SUR, sulfonylurea receptor; VCR, video recorder; Vm, voltage membrane

Introduction

Taurine is a sulphonic aminoacid widely distributed in different tissues involved in many physiological processes such as osmoregulation, antioxidant action, and control of Ca^{2+} homeostasis (Huxtable & Sebring, 1986).

There is emerging evidence that the mechanism of action responsible for the physiological effects of taurine involving two factors is complex: first, the combination of the actions of the sulphonic aminoacid on several types of ion channels, transporters and enzymes; second, the environmental conditions. For example, in cardiac cells the effects of taurine are Ca^{2+} dependent, being an agonist of the Ca^{2+} current and delayed rectifier K^+ current at low internal Ca^{2+} concentration (10^{-8} M) in turn promoting the shortening of the action potential duration (APD) with an inotropic effect (Satoh & Horie, 1997; Satoh, 1998). Conversely, at high Ca^{2+} ion concentration (10^{-6} M), taurine inhibits both types of currents resulting in the prolongation of the APD. This phenomenon in conjuction with the inhibition of the cardiac fast Na^+ current contributes to the observed anti-ischaemic effect of the sulphonic aminoacid (Schanne & Dumaine, 1992; Satoh, 1998). Thus taurine might be expected to exert inotropic effects or anti-ischaemic effects depending on the internal concentration of the Ca^{2+} ion (Satoh, 1998). The anti-ischaemic effects of taurine are also supported by the fact that during cardiac ischaemia the intracellular content of the sulphonic aminoacid decreases (Kramer *et al*., 1981; Saransari & Oja, 1998). It has been proposed that, in cardiac fibres, this phenomenon would promote activation of a class of K^+ channel the ATP-sensitive K^+ channel (K_{ATP}) that is inhibited by taurine in a millimolar concentration range (Satoh, 1996). This is of certain importance, since the sarcolemma K_{ATP} channel opens in response to ischaemia insults leading to the repolarization of the fibres, reduction of the influx of Ca^{2+} ion, thus saving the intracellular ATP content, and in turn promoting cytoprotective effects (McPherson *et al*., 1993; Hearse, 1995). This idea is supported by the fact that K_{ATP} channel-deficiency COS-7 cells, which are vulnerable to chemical hypoxia-reoxygenation injury, when co-transfected with the cardiac K_{ATP} channel subunits and in the presence of agonists (KCO), gain resistance to hypoxia-reoxygenation injury (Jovanovic *et al*., 1998). A cytoprotective role of the K_{ATP} channel against ischaemia has also been proposed in the skeletal muscle, in which the openings of the muscular K_{ATP} channel by agonists appear to be involved in the anti-infarction effect of the ischaemic preconditioning (Pang *et al*., 1997).

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However, to date the effects of taurine on skeletal muscle K_{ATP} channels are not known. Our previous work showed that taurine in skeletal muscle plays a fundamental role in the electrical stabilization of the sarcolemma through an increase of the macroscopic chloride conductance (GCI) (Conte Camerino *et al*., 1987). This is more clearly demonstrated by the reduction of GCI and by the shift to negative potentials of the voltage threshold for mechanical contraction consequent to experimentally induced taurine depletion (De Luca *et al*., 1996). In addition, taurine can counteract the low GCI in some forms of pharmacologically induced myotonias in rats (Conte Camerino *et al*., 1989) and in the age-dependent decrease of GCI (Pierno *et al*., 1998).

Furthermore, the mechanism by which taurine affects the K_{ATP} channel has not been extensively investigated. For example, it is not known whether the aminoacid affects the K_{ATP} channel by a mechanism which is dependent on the functional state of the channel complex (e.g. before and after rundown), since the effects of the K_{ATP} channel modulators depend on the functional state of the channel (Terzic, 1995; Tricarico *et al*., 1998a). This is further complicated by the fact that splice variants of the sulphonylurea receptor (SUR), when assembled with the inward rectifier K^+ channel (Kir) forming the K_{ATP} channel complex (Yokoshiki *et al*., 1998), are expressed in the skeletal muscle contributing to the variability of the responses of the channel to different modulators (Chutkov *et al*., 1999).

In the present work we evaluated the effect of taurine on skeletal muscle K_{ATP} channels on macropatches immediately after excision or several minutes (on rundown channels) after patch excision. In order to study the interaction of taurine with K_{ATP} channels, we performed competition experiments constructing dose-response curves of specific blockers of these channels such as ATP and glybenclamide, and of channel modulator, the Ca^{2+} ion, in the presence of taurine. Single channel recordings were also performed to evaluate the possible effects of taurine on the channel gating.

Methods

Isolation of single fibres

Single fibres were prepared from flexor digitorum brevis (FDB) muscles of male adult Wistar rats of 5–6 months of age by enzymatic treatment as previously described (Tricarico & Conte Camerino, 1994).

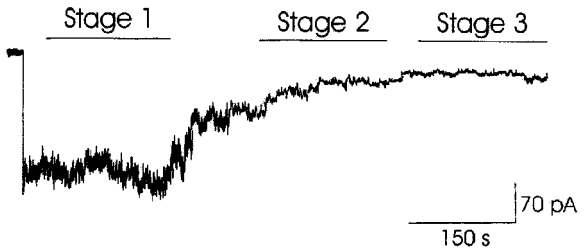

Figure 1 Time-dependent decay of the K_{ATP} current in an excised macropatch having a pipette area of 6.1 μm^2. Sample trace of continuous recording of K_{ATP} current performed, at −60 mV (Vm), on inside-out patch with 150 mM KCl on both sides of the membrane. The background current was subtracted. The decay of the current can be described by three stages of different amplitude. Upon excision the current remained stable for 4 min (stage 1, time frames 20 s–4 min) then slowly decayed reaching a new level of lower amplitude (stage 2, time frames 7 min–10 min). After 12 min the patch became almost silent (stage 3).

Electrophysiology

Experiments were performed on inside-out membrane patches using standard patch-clamp techniques. Recordings of K_{ATP} current (I) were performed on macropatches during voltage steps from 0 mV (holding potential) to −60 mV with 150 mM KCl on both sides of the membrane patches, at 20°C. The spontaneous time-dependent decay of the current was observed at a constant voltage of −60 mV. The currents, sampled at 5 kHz and filtered at 0.5 kHz, were video taped by using a VCR system and played back later for computer analysis (Tricarico *et al*., 1998a). Macropatch currents were recorded by using Axon hardware and pClamp software (Tricarico & Conte Camerino, 1994). The effects on channels under similar conditions were determined, at −60 mV, immediately after excision, 6±2 min ($n=21$) (early stage of rundown) and 11±3 min ($n=21$) (late stage of rundown) after patch excision and in the absence of ATP.

Competition experiments were performed constructing dose-response curves of different antagonists of the K_{ATP} channel in the presence of taurine. In particular, the effects of taurine (10 mM) on macropatch currents were evaluated immediately after excision in the absence or in the presence of internal increasing concentrations of ATP (1–500 μM), glybenclamide (0.2–100 nM) or Ca^{2+} ion (0.2–32 μM).

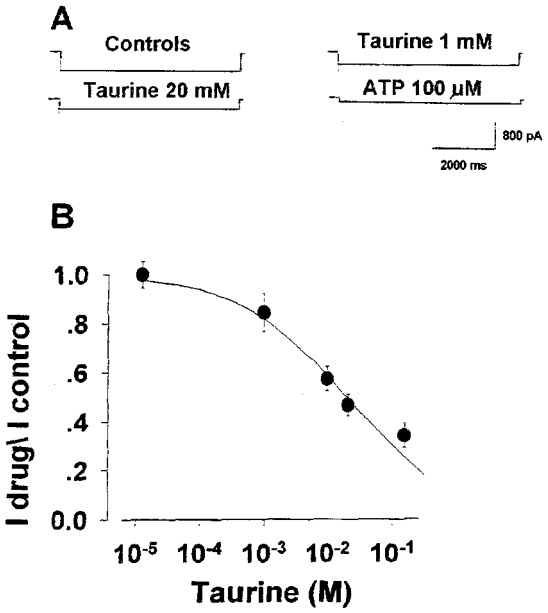

Figure 2 Effects of taurine on K_{ATP} currents of rat skeletal muscle recorded immediately after excision (stage 1). Digital average of K_{ATP} current traces from 21 patches recorded at −60 mV (Vm), in inside-out configuration, immediately after patch excision. The background currents were subtracted. (A) K_{ATP} currents of −566±29 pA of amplitude recorded in the presence of 150 mM KCl on both sides of the membranes. In the presence of 1 mM concentration of taurine the current amplitude was −388±27 pA. At 20 mM concentration the sulphonic aminoacid reduced the current at −311±12 pA of amplitude. The internal application of ATP, at 100 μM concentration, fully reduced the current at −111±12 pA of amplitude confirming that it was sustained by K_{ATP} channels. (B) Dose–response curve of the K_{ATP} current of rat skeletal muscle fibres versus taurine concentrations. The ordinate represents the ratio between the K_{ATP} currents in the presence of taurine and the currents in the absence of the drug (I compound/I control). The abscissa represents the concentrations of taurine. The sulphonic aminoacid inhibited the currents dose-dependently. Each experimental point represents the mean±s.e. of a minimum of five and a maximum of six macropatches.

Pipettes were prepared as previously described (Tricarico & Conte Camerino, 1994). Macropipettes having an average tip opening area of 4.9 ± 0.1 μm^2 ($n = 44$) were used to measure the current sustained by multiple channels and the pharmacological properties of the K_{ATP} channel.

The single channel conductance and kinetics were measured by using micropipettes having a tip opening area of 0.85 ± 0.03 μm^2 ($n = 21$). Having used this type of pipette, no more than 2–3 open channels were observed in the patches. Few patches contained a single active channel.

Analysis

The current (I) flowing through the macropatches was calculated by subtracting the baseline level of the current (defined as the closed state of the channels and measured in the presence of ATP) from the open channel level. The concentration–response relationships could be described by the following equation:

$$\text{I drug/I control} = 1/(1 + ([\text{Drug}]/\text{IC}_{50})^{n}) \qquad (1)$$

where I drug/I control is the ratio between the current measured in the presence and in the absence of taurine; IC_{50} is the concentration of taurine needed to reduce the current by 50%; n is the Hill coefficient of the curves; [Drug] is the taurine concentration. The algorithms of the fitting procedures used were based on the Marquardt least-squares fitting routine.

The single channel conductance and the kinetic parameters, the open probability (P_{open}), the burst durations and the close intervals between bursts were calculated as previously described (Tricarico *et al*., 1998a,b). The overall open probability (P_{open}) was measured as the ratio between the time spent in the open state and the total time of recording. Kinetic analysis was performed within the bursts of opening when single channel was active in the patches (Tricarico *et al*., 1998a). The open and close time distributions within the bursts before and after rundown were fitted with the sum of two exponential functions as previously described (Tricarico *et al*., 1998a).

Significant differences between individual pairs of means were determined by using the paired Student *t*-test. The data is expressed as mean ± s.e.

Drugs and solutions

The solutions had the following composition (in mM): Pipette, KCl 150, $CaCl_2$ 2, 3-(N-morpholino)propanesulphonic acid (MOPS) 10, pH = 7.2; Bath, normal Ringer NaCl 145, KCl 5.5, $MgCl_2$ 1, $CaCl_2$ 0.5, glucose 5, MOPS 10, pH = 7.2; symmetrical K^+ KCl 150, ethylene glycol-bis(b-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) 0.5, MOPS 10, pH = 7.2. Stock solutions (5 mM) of adenosine-triphosphate sodium salt (Na_2ATP), and taurine (400 mM) were prepared by dissolving the chemicals in the symmetrical K^+ solution. Glybenclamide was first dissolved in dimethylsulphoxide at a concentration of 1 mg ml^{-1}. Aliquots of these solutions were added to the bath solution to give the concentrations required. Free Ca^{2+} ion concentrations in the bath solution ranging between 0.2 and 32 μM were prepared as previously described (Tricarico *et al*., 1997). The possible influence of the osmolarity on the K_{ATP} channel activity was evaluated by applying a bath solution enriched with saccarose to the internal side of the patches. As previously shown (Tricarico *et al*., 1997), no significant effects were observed after the addition to the bath of a solution containing 20 and 60 mM sucrose.

Results

Effect of taurine on K_{ATP} channel immediately after excision

A large K_{ATP} current was elicited immediately by excision of the macropatches (pipette area = 4.9 ± 0.1 μm^2) from the fibres into an ATP free solution. The internal application of ATP abolished the current confirming that K_{ATP} channels sustained it. The effects of taurine were examined in the time frames of 20 s–3 min from patch excision called stage 1 (Figure 1). Indeed, as previously shown (Tricarico *et al*., 1998a), after 4 ± 1.7 min from excision the K_{ATP} current started to rundown (Figure 1). At −60 mV (Vm), in the presence of 150 mM KCl on both sides of the membrane and in the absence of ATP (100 μM) the current had an average amplitude of -252.3 ± 32 pA (n patches = 22) per patch area. We found that the application of taurine (1–100 mM) to the patches induced a dose-dependent reduction of the K_{ATP}

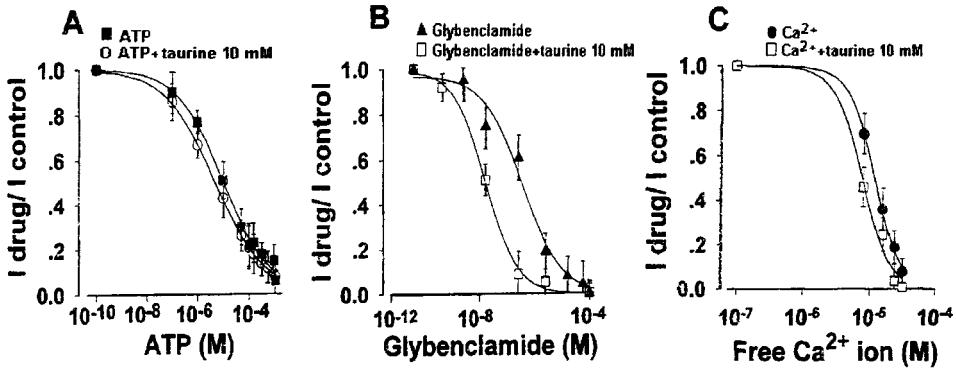

Figure 3 Dose–response curves of K_{ATP} current of rat skeletal muscle fibres versus ATP (A) glybenclamide (B) and Ca^{2+} ion (C) concentrations in the absence or in the presence of taurine (10 mM). The ordinate represents the ratio between the K_{ATP} currents in the presence of the blocker and the currents in the absence of the blocker (I compound/I control). The abscissa represents the concentrations of the blocker. The K_{ATP} current was recorded at −60 mV (Vm), in inside-out configuration, immediately after patch excision. Taurine shifted the dose-response curve of glybenclamide to the left on the log–dose axis without significantly affecting those of ATP and Ca^{2+} ion. Each experimental point is the mean ± s.e. of a minimum of six and a maximum of nine macropatches.

current that was restored after washout of the drug solution (Figure 2A). The concentration of taurine needed to produce the half inhibition of the current was 20.1 ± 6 mM (slope = 0.51). At the highest concentration tested (100 mM) taurine did not completely reduce the K_{ATP} current (Figure 2B). Experiments were performed to evaluate the possible interaction of taurine with the binding sites of classical channel blockers such as ATP and glybenclamide. In the absence of taurine, ATP inhibited K_{ATP} currents dose-dependently with an IC_{50} of 8.1 ± 0.3 μM (slope = 0.65) (Figure 3A) (n patches = 7). In the presence of taurine (10 mM) the IC_{50} value for ATP was 5.9 ± 0.5 μM (slope = 0.45) (Figure 3A) (n patches = 9). Under these experimental conditions washout restored the current.

As expected, the internal application of glybenclamide inhibited the K_{ATP} current dose-dependently. Taurine caused a leftward and significant shift of the dose–response curve of glybenclamide (Figure 3B). The IC_{50} was 50 ± 6 nM (slope = 0.41) for gybenclamide alone (n patches = 6) and 20 ± 3 nM (slope = 0.68) for glybenclamide in the presence of taurine (10 mM), respectively ($P > 0.001$) (n patches = 9).

The internal application of different concentrations of free Ca^{2+} ions to the macropatches reduced the K_{ATP} currents, with an IC_{50} of 11.8 ± 2 μM (slope = 2.17) (n patches = 7). In the

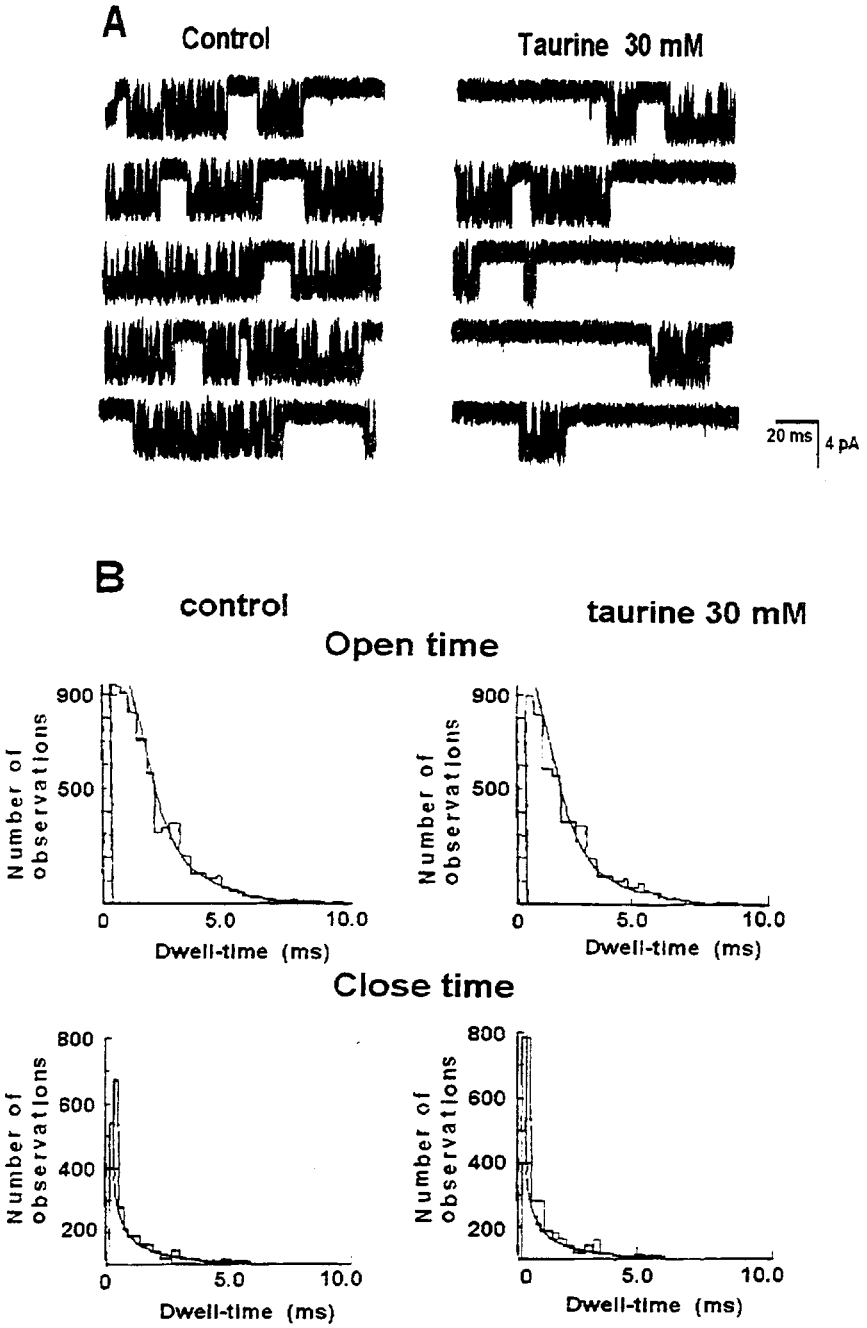

Figure 4 Effects of taurine on a single K_{ATP} channel immediately after excision. Downward deflections in the current record indicate inward current. (A) Continuous recording of channel activity was performed few seconds after excision, at −60 mV (Vm), with 150 mM KCl on both sides of the membrane in the absence (control) and in the presence of 30 mM concentration of taurine. The sulphonic aminoacid decreased the burst duration and prolonged the close time intervals within the bursts. (B) Kinetic analysis of the channel traces in A, performed within the bursts of openings. As shown, the open and close time distributions were fitted by two exponential functions not modified by taurine.

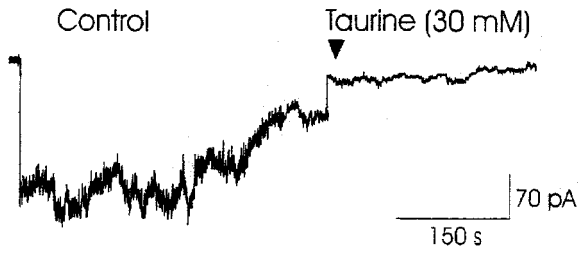

Figure 5 Effects of taurine on K_{ATP} currents during rundown (stage 2). The current was recorded by a macropatch having a tip opening area of 6 μm^2, at -60 mV (Vm), in the presence of 150 mM KCl on both sides of the membrane. The background current was subtracted. The internal application of 30 mM concentration of taurine to the patch reduced the current by about 60% with respect to that measured in the absence of the sulphonic aminoacid and after rundown.

presence of taurine (10 mM), the concentration–response curve of the K_{ATP} currents versus Ca^{2+} concentrations was not significantly modified showing an IC_{50} of 8.95 ± 3 μM (slope = 2.07) (n patches = 9) (Figure 3C).

Single channel current was recorded by micropatches (pipette area = 0.85 ± 0.03 μm^2), at -60 mV (Vm), in the absence and in the presence of 30 mM concentration of taurine. The sulphonic aminoacid reduced the channel open probability by 55.7%. In particular, the P_{open} decreased from 0.411 ± 0.02 in the controls to 0.182 ± 0.04 (n patches = 3) in the presence of taurine. This effect was mediated by the reduction of the mean burst duration which was 48.1 ± 6 ms in the controls and 22.2 ± 5 ms (n patches = 3) in the presence of taurine (Figure 4A). The sulphonic aminoacid also prolonged the close time intervals separating the bursts of openings, increasing it from 71 ± 12 ms in the controls to 151 ± 29 ms in the presence of taurine (n patches = 3) (Figure 4A).

Kinetic analysis performed within the bursts of openings revealed that taurine did not alter the open and close time distributions. The open dwell-time distributions was well fitted by the sum of two exponential functions showing $\tau 1$ and $\tau 2$ values of 0.62 ± 0.06 and 2.15 ± 0.7 ms (n patches = 3), respectively, in the controls, and 0.59 ± 0.05 and 2.21 ± 0.09 ms, respectively, in the presence of 30 mM concentration of taurine (Figure 4B). The close dwell-time distribution was well fitted by the sum of two exponential functions showing $\tau 1$ and $\tau 2$ of 0.34 ± 0.04 and 0.21 ± 0.02 ms, respectively, in the controls, and 0.33 ± 0.06 and 0.19 ± 0.04 ms, respectively, in the presence of the sulphonic aminoacid (Figure 4B).

Taurine did not affect the unitary conductance of the K_{ATP} channel. The slope conductance measured in the negative range of potentials was 72 ± 4 pS (n patches = 3) in the controls and 69 ± 6 pS in the presence of 30 mM concentration of taurine, whereas in the positive range of potentials it was 40 ± 6 pS (n patches = 3) in the controls and 38 ± 8 pS in the presence of the sulphonic aminoacid.

Effect of taurine on K_{ATP} channel after rundown

The effects of taurine were also evaluated on the K_{ATP} current in the time frames of 7–12 min from patch excision in stages 2 and 3 (Figure 1). After about 7 min from excision, the currents, at -60 mV (Vm), in the absence of taurine, reached a new value of 75 ± 8 pA ($n = 20$) being also sensitive to the stimulatory effect of the nucleoside di-phosphates. In this experimental condition, the application of 30 mM concentration of taurine to the macropatches reduced the currents by 57% (Figure 5) (n patches = 4), an effect similar to that observed on channels immediately after excision (Figure 2E).

Single channel recordings were performed, at -60 mV (Vm), after 8 min from excision in the absence and in the presence of 30 mM concentration of taurine. The application of taurine to the excised patches caused a reduction of the channel open probability by 54%, being 0.081 ± 0.03 (n patches = 4) in the controls and 0.037 ± 0.012 in the presence of the sulphonic aminoacid. In the same patches, the sulphonic aminoacid reduced the mean burst duration from 18.1 ± 7 ms in the controls to 8.1 ± 5 ms (n patches = 4) in the presence of taurine. The sulphonic aminoacid also increased the close time intervals separating the bursts of openings by 53%, being 1520 ± 421 ms in the controls and 3211 ± 511 ms in the presence of taurine (n patches = 4) (Figure 6A). No significant change was observed in the open and close time distributions after rundown in the presence of taurine. The $\tau 1$ and $\tau 2$ of the open dwell-time distributions were 0.48 ± 0.07 ms and 1.71 ± 0.09 ms, respectively, in the controls (n patches = 4), and were 0.46 ± 0.05 ms and 1.88 ± 0.08 ms, respectively, in the presence of taurine (Figure 6B). The $\tau 1$ and $\tau 2$ of the close dwell-time distribution were 0.29 ± 0.05 and 0.19 ± 0.04 ms, respectively, in the controls (n patches = 4), and 0.27 ± 0.06 and 0.18 ± 0.06 ms, respectively, in the presence of taurine (Figure 6B).

Taurine did not affect the unitary conductance of the K_{ATP} channel. The slope conductance measured in the negative range of potentials was 68 ± 6 pS (n patches = 4) in the controls and 67 ± 8 pS in the presence of 30 mM concentration of taurine, whereas in the positive range of potentials it was 38 ± 6 pS (n patches = 4) in the controls and 41 ± 8 pS in the presence of the sulphonic aminoacid.

Discussion

We found that taurine behaves as an inhibitor of skeletal muscle K_{ATP} channels showing an IC_{50} of 20 mM. This is not surprising considering that the effects of taurine on several ion channels and transporters are significant in the millimolar concentrations, and that in most tissues the intracellular content of the sulphonic aminoacid ranges between 10 and 60 mM (Pasantes-Morales *et al.*, 1998). In cardiac tissue taurine inhibited the K_{ATP} channel with an IC_{50} of 13.5 mM (Han *et al.*, 1996; Satoh, 1996) which is lower as compared to that calculated in the skeletal muscle in our experiments. At least two mechanisms may explain the different responses of the skeletal and cardiac muscle K_{ATP} channels to taurine. First, splice variants of the SUR subunit possibly showing a different pharmacological profile are expressed in the cardiac and skeletal muscles contributing to the variability of the responses of the channels to taurine (Chutkow *et al.*, 1999). Second, in our experiments the dose–response curves were constructed in the presence of multi-channel preparations, whereas in the cardiac cells the experiments were performed on a single unit (Han *et al.*, 1996; Satoh, 1996). This appears to be an important factor in patch clamp experiments in which channel–channel interactions induce a negative cooperativity phenomenon reducing the sensitivity of the channel to the blockers (Tricarico *et al.*, 1998a).

Attempts were made to investigate the mechanism of action of taurine on the muscular K_{ATP} channel. The fact that the dose-response curves of the K_{ATP} current versus ATP and Ca^{2+} ion concentrations did not significantly differ in respect to that constructed in the presence of taurine alone, indicates that the sulphonic aminoacid does not interfere with the binding sites for ATP and Ca^{2+} ion and therefore its effects are not ATP and

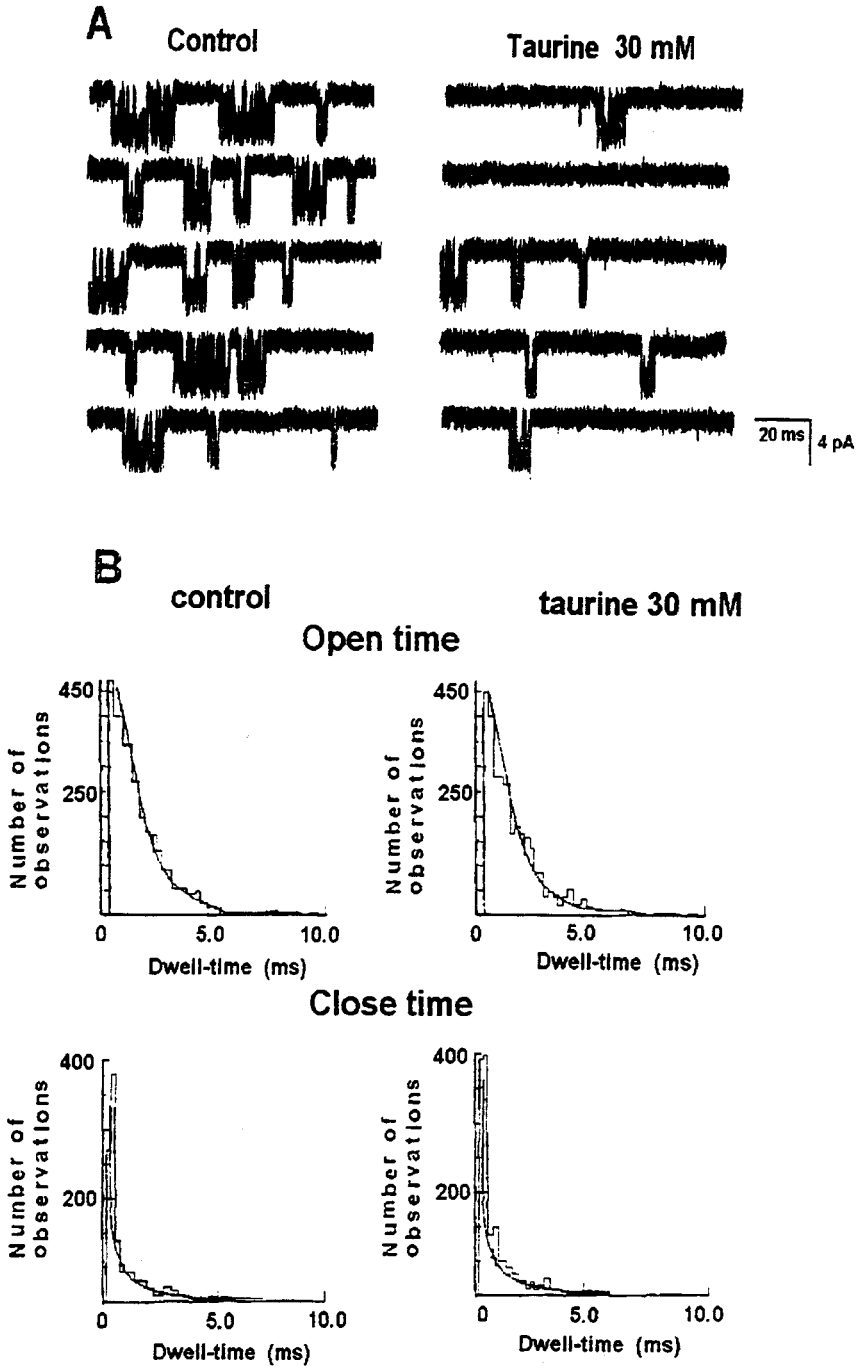

Figure 6 Effects of taurine on a single K_{ATP} channel after rundown. Downward deflections in the current record indicate inward current. (A) Continuous recording of channel activity was performed after 8 min from excision, at −60 mV (Vm), with 150 mM KCl on both sides of the membrane in the absence (control) and in the presence of 30 mM concentration of taurine. The sulphonic aminoacid decreased the burst duration and prolonged the close time intervals within the bursts. (B) Kinetic analysis of the channel traces in A, performed within the bursts of openings. As shown, the open and close time distributions were fitted by two exponential functions not modified by taurine.

Ca^{2+} dependent. Chimera construct studies locate the ATP site on the carboxyterminus of the Kir subunit of the K_{ATP} channel complex (Drain *et al*., 1998), which also appears to be the site for the Ca^{2+} ion that would bind to the interface between the cytosolic loops containing the ATP site of the Kir (Trap *et al*., 1997; Drain *et al*., 1998) and the membrane phospholipids forcing the pore into the non conductive state (Fan & Makielski 1997). In contrast, we found that the sulphonic aminoacid potentiated the inhibitory effect of glybenclamide on the K_{ATP} channel. This can be due to an interaction of taurine with a site located on SUR proteins, or alternatively, taurine may affect the K_{ATP} channel by interacting with a site allosterically coupled to the SUR. Our data support the hypothesis that taurine allosterically modifies the K_{ATP} channel by binding to the polar phase of the membrane phospholipids on sites closely related to the SUR protein, in turn affecting the channel gating. We come to this conclusion considering that in our experiments the mechanism of action of the sulphonic aminoacid appears to be independent of the functional state of the channel being indeed dependent on the channel gating. This is demonstrated by the

fact that the effects of taurine on both the macroscopic current and single channel current, measured immediately after patch excision as well as after rundown, do not differ significantly. Whereas, taurine affects the close state of the channel before and after rundown. The fact that the effects of taurine are independent from the functional state of the K_{ATP} channel appears to be a unique property of the sulphonic aminoacid; in fact, it is known that several agonists and antagonists of the K_{ATP} channel, having specific binding sites on the protein phase of SUR, also show effects which are dependent on the functional state of the channel (Schwanstecher *et al*., 1998). For example, the action of the sulphonylureas on the K_{ATP} channel depends on the operative condition of the channel, being strong inhibitors of cardiac channel at the beginning of ischaemia-poisoning and losing the potency during channel rundown (Findlay, 1992; Brady *et al*., 1998). Similarly we have found that mexiletine a well known anti-arrhythmic drug of Ib class, blocks skeletal muscle K_{ATP} channels by a mechanism of action that depends on the functional state of the channel being more effective on channels immediately after excision and losing the potency after rundown (Tricarico *et al*., 1998a). It is a common idea that the different responses that the K_{ATP} channel shows to the same molecule in various experimental conditions are not related to the chemical structure of the compound, but it depends on the structure and function of the K_{ATP} channel complex (Terzic *et al*., 1995).

In conclusion, the physiological effect of taurine in skeletal muscle appears to be a combination of multiple effects. For example, the activation of Cl^- channels in skeletal muscle, and the inhibition of Na^+ and Ca^{2+} channels in heart muscles caused by taurine, can allow a rapid fibre repolarization and savage the energy store of the cells. In the ischaemic and working muscle, these effects can also be sustained by the early openings of the K_{ATP} channel provoked by the decrease of the intracellular taurine content (Kramer *et al*., 1981; Saransari & Oja, 1998).

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