HAL
open science

A large-conductance calcium-activated potassium channel in potato tuber mitochondria

Izabela Koszela-Piotrowska, Karolina Matkovic, Adam Szewczyk, Wieslawa Jarmuszkiewicz

To cite this version:

Izabela Koszela-Piotrowska, Karolina Matkovic, Adam Szewczyk, Wieslawa Jarmuszkiewicz. A large-conductance calcium-activated potassium channel in potato tuber mitochondria. Biochemical Journal, 2009, 424 (2), pp.307-316. 10.1042/BJ20090991 . hal-00479217

HAL Id: hal-00479217

https://hal.science/hal-00479217v1

Submitted on 30 Apr 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

A Large-Conductance Calcium-Activated Potassium Channel in Potato Tuber Mitochondria

Izabela KOSZELA-PIOTROWSKA[#,a], Karolina MATKOVIC[#,b], Adam SZEWCZYK[a,1] and Wieslawa JARMUSZKIEWICZ[b]

[a]Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, 3 Pasteur st., 02-093 Warsaw, Poland, [b]Laboratory of Bioenergetics, Faculty of Biology, Adam Mickiewicz University, Poznan, Poland

Short title: Calcium-activated potassium channel in plant mitochondria

[#] These authors contributed equally to this work

To whom correspondence should be addressed: Adam Szewczyk, Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw, Poland, Tel.: +48225892269; Fax: +48228225342; E-mail: adam@nencki.gov.pl

Abbreviations used: BK_{Ca} channel, plasma membrane large-conductance Ca^{2+}-activated potassium channel; BSA, bovine serum albumin; IbTx, iberiotoxin; KCOs, potassium channel openers; mitoBK_{Ca} channel, mitochondrial large-conductance Ca^{2+}-activated potassium channel; mitoK_{ATP} channel, mitochondrial ATP-sensitive potassium channel; NS1619, 1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H-benzimidazole-2-one; PLM, planar lipid membrane; SMP, submitochondrial particles; TPP^+, tetraphenylphosphonium $\Delta\Psi$, mitochondrial transmembrane electrical potential

Key words: bioenergetics; electrophysiology; mitochondria; potassium channel; *Solanum tuberosum*;

THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/BJ20090991

Accepted Manuscript

Licenced copy. Copying is not permitted, except with prior permission and as allowed by law.
© 2009 The Authors Journal compilation © 2009 Portland Press Limited

Synopsis
In the present study, we describe the existence of a novel potassium channel in the plant (potato tuber) mitochondrial inner membrane. We found that substances known to modulate large-conductance calcium-activated potassium channel activity influenced the bioenergetics of potato tuber mitochondria. In isolated mitochondria, Ca^{2+} and NS1619 (a potassium channel opener) were found to depolarize the mitochondrial membrane potential and to stimulate resting respiration. These effects were blocked by iberiotoxin (a potassium channel inhibitor) in a potassium-dependent manner. Additionally, the electrophysiological properties of the large-conductance potassium channel present in the potato tuber inner mitochondrial membrane are described in a reconstituted system, using planar lipid bilayers. After incorporation in 50/450 mM KCl gradient solutions, we recorded large-conductance potassium channel activity with conductance from 502 ± 15 to 615 ± 12 pS. The probability of channel opening was increased by Ca^{2+} and reduced by iberiotoxin. Immunological analysis with antibodies raised against the mammalian plasma membrane large-conductance Ca^{2+}-dependent K^+ channel identified a pore-forming α subunit and an auxiliary β2 subunit of the channel in potato tuber mitochondrial inner membrane. These results suggest that a large-conductance calcium-activated potassium channel similar to that of mammalian mitochondria is present in potato tuber mitochondria.

THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/BJ20090991

Accepted Manuscript

Licenced copy. Copying is not permitted, except with prior permission and as allowed by law.
© 2009 The Authors Journal compilation © 2009 Portland Press Limited

THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/BJ20090991

INTRODUCTION

Mitochondria play an important role in energy metabolism within the cell. In addition to this canonical function, mitochondria are involved in intracellular signaling. Recently, potassium transport through the mitochondrial inner membrane was found to play a central role in the cytoprotection of various mammalian cell types [1,2]. This transport is strictly ion channel-dependent. Similarly to the plasma membrane, potassium-selective ion channels are found in the inner mitochondrial membrane [3-5]. Potassium ions control mitochondrial metabolism, primarily via a regulation of matrix volume [6]. The biophysical and pharmacological properties of mitochondrial potassium channels are similar to some of the potassium channels present in the plasma membrane of various mammalian cell types, for example mitochondrial ATP-regulated potassium channels (mitoK_{ATP} channels) [7], large-conductance calcium-activated potassium channels (mitoBK_{Ca} channels) [8], and voltage-dependent potassium Kv1.3 channels [9].

In plant mitochondria, the existence of different energy-dissipating systems is necessary for metabolic regulation [10]. Plant mitochondria (but not animal mitochondria) contain an alternative oxidase (AOX) that catalyses ubiquinol-oxygen oxidoreduction without H^+ release into the cytosol, thus dissipating the redox potential energy [11,12]. Similarly to animal mitochondria, plant mitochondria contain an uncoupling protein (PUMP) that dissipates a proton electrochemical gradient by mediating re-entry of H^+ into the mitochondrial matrix in the presence of free fatty acids [13]. Another energy-dissipating system present in plant mitochondria (as in animal mitochondria) is the ATP-sensitive potassium channel (mitoK_{ATP} channel), which mediates an electrophoretic K^+ uniport, likely working together with an electroneutral K^+/H^+ exchanger [14-17]. Descriptions of the plant mitoK_{ATP} channel have been based mainly on studies of isolated mitochondria in which the influence of K^+ entry into the mitochondrial matrix on membrane potential ($\Delta\Psi$) dissipation and mitochondrial swelling were assessed. The plant mitoK_{ATP} channel seems to be sensitive to the same potassium channel modulators as the mammalian mitoK_{ATP} channel. In durum wheat and pea mitochondria, the mitoK_{ATP} channel is activated by diazoxide and GTP, and its activity is inhibited by glyburide and 5-hydroxydecanoate (in pea mitochondria) [14,16,18]. Moreover, the plant mitoK_{ATP} appears to be stimulated by cyclosporin A and regulated by dithiol-disulfide interconversion, H_2O_2, and NO [15,16,19]. On the other hand, an ATP-insensitive, quinine-inhibited potassium import pathway has been described in potato, tomato, and maize mitochondria [20].

So far, there has been no information about other possible plant mitochondrial potassium channels, such as the large-conductance Ca^{2+}-activated potassium channel (mitoBK_{Ca} channel) described in animal mitochondria. Therefore, the aim of this study was to search for a mitoBK_{Ca} channel in plant mitochondria and to determine the bioenergetic consequences of its activation. For this purpose, we studied the effects of BK_{Ca} channel activators (Ca^{2+}, NS1619) and inhibitor (iberiotoxin, IbTx) on $\Delta\Psi$ and respiration in isolated potato tuber mitochondria. Moreover, measurements of the electrophysiological properties of a single mitoBK_{Ca} channel in a reconstituted system were carried out and immunological detection experiments were performed to confirm the discovery of a plant mitoBK_{Ca} channel.

EXPERIMENTAL

Chemicals

L-α-phosphatidyl-choline (asolectin), NS1619, and *n*-decane were purchased from Sigma-Aldrich, and IbTx from Bachem. All other chemicals were of the highest purity commercially available. NS1619 was dissolved in methanol, and IbTx in H_2O.

Isolation of mitochondria

Potato (*Solanum tuberosum*) tubers were purchased from the local supermarket. One kilogram of peeled potato tubers were homogenized in a blender in 1 liter of ice-cold medium consisting of 0.35 M mannitol, 3 mM EDTA, 3 mM EGTA, 6 mM cysteine, 0.1% bovine serum albumin (BSA), and 25 mM NaH_2PO_4/ Na_2HPO_4 buffer. The pH was adjusted to 8.0 with NaOH. The crude extract was quickly filtered through cheesecloth, and the filtrate was centrifuged at 1000 x g for 10 min. The supernatant was further centrifuged at 11000 x g for 20 min. The pellet of crude mitochondria was washed with medium consisting of 0.35 M mannitol, 1.5 mM EGTA, 0.1% BSA, and 10 mM NaH_2PO_4/Na_2HPO4 buffer (pH 6.8) and centrifuged

Licenced copy. Copying is not permitted, except with prior permission and as allowed by law.
© 2009 The Authors Journal compilation © 2009 Portland Press Limited

Accepted Manuscript

again at 1000 x g for 5 min and then at 11000 x g for 20 min. Mitochondria were subsequently purified on a self-generating 22% Percoll gradient (at 40000 x g for 30 min). Purified mitochondria were washed twice in the same medium without EGTA and BSA. The mitochondrial protein concentration was determined by the biuret method.

Preparation of membrane fractions

To obtain submitochondrial particles (SMP), mitochondria were isolated as above, but with media of K^+ salts. Freshly prepared mitochondria were diluted to about 8 mg of protein/ml with 0.3 M sucrose and 10 mM Hepes-KOH, pH 7.2, and subsequently frozen at -20ºC. After thawing, the suspension was sonicated 6 times for 30 s and centrifuged at 12000 × g for 15 min to pellet the unbroken mitochondria. The supernatant was centrifuged at 200000 × g (2 h) to pellet SMP. Fractions of SMP, which are the mitochondrial inner membrane-enriched fractions, were resuspended in 0.3 M sucrose and 10 mM Hepes-KOH, pH 7.2 at a concentration of 5 mg of protein/ml.

To obtain a plasma membrane-enriched fraction, the supernatant from the first 11000 x g centrifugation during mitochondria isolation was centrifuged at 100000 x g for 1h. The pellet was resuspended in 0.3 M sucrose and 10 mM Hepes-KOH, pH 7.2.

Planar lipid membrane (PLM) measurements

Experiments were performed with potato tuber SMP, as described previously [21-23]. In brief, PLMs were formed in a 250-µm-diameter hole drilled in a Delrin cup (Warner Instrument, CT), which separates the two chambers (*cis* and *trans*, each with a 1-ml internal volume). The chambers contained 50/450 mM KCl (*trans/cis*) and 20 mM Tris-HCl solutions, pH 7.2. The outline of the aperture was coated with a lipid solution and N_2 dried prior to bilayer formation to improve membrane stability. PLMs were painted using asolectin in *n*-decane at a final concentration of 25 mg of lipid/ml. SMP (about 3 µg of protein/ml, 0.5-1.5 µl/reconstitution) were added to the *trans* compartment (Figure 5A). All measurements were carried out at room temperature (~25ºC). Formation and thinning of the bilayer were monitored by capacitance measurements and optical observations. Final accepted capacitance values ranged from 120 to 230 picofarads. Electrical connections were created using Ag/AgCl electrodes and agar salt bridges (3 M KCl) to minimize liquid junction potentials. Voltage was applied to the *cis* compartment of the chamber, and the *trans* compartment was grounded. The current was measured using a bilayer membrane amplifier (BLM-120, BioLogic). Signals were low-pass filtered at 500 Hz. The current was digitized at a sampling rate of 100 kHz (A/D converter PowerLab 2/20, ADInstruments) and transferred to a PC for off-line analysis by Chart version 5.5.5 (PowerLab ADInstruments) and pCLAMP10.2 (Axon Instruments). The pCLAMP10.2 software package was used for data processing. The channel recordings presented are representative of the most frequently observed conductance values under the given conditions. Ion conductance was calculated from the linear regression of the points in the current-voltage relationship using the GraphPad Prism 4 program.

Mitochondrial oxygen consumption

Oxygen uptake was measured polarographically with a Clarck type oxygen electrode (Rank Brothers) in 3 ml of standard incubation medium (25°C) consisting of 0.35 M mannitol, 3 mM NaH_2PO_4, 1 mM $MgCl_2$, 10 mM TrisHCl, 10 mM MES, pH 6.8, and 0.1% BSA. Changes in the composition of the incubation medium are described in the figure legend. Measurements were performed with 0.8-1 mg of mitochondrial protein in the presence of 1.5 mM benzohydroxymate (an inhibitor of alternative oxidase), 0.14 mM ATP (to activate succinate dehydrogenase), 1.8 µM carboxyatractylozide (to exclude ATP/ADP antiporter activity), and 10 mM glibenclamide (to inhibit the mitoK_{ATP} channel). Succinate (5 mM) plus rotenone (2 µM) was used as a respiratory substrate. Respiratory rate measurements were performed in the absence of added ADP, *i.e.*, in the resting state (state 4). State 3 (phosphorylating) respiration measurements were performed to check the coupling parameters. Only high quality mitochondria preparations, *i.e.*, with an ADP/O value of around 1.40 and a respiratory control ratio of around 2.5-3 (with succinate), were used in all experiments. O_2 uptake values are presented in nmol O × min^{-1} × mg^{-1} protein.

THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/BJ20090991

Accepted Manuscript

Licenced copy. Copying is not permitted, except with prior permission and as allowed by law.
© 2009 The Authors Journal compilation © 2009 Portland Press Limited

THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/BJ20090991

Accepted Manuscript

Mitochondrial membrane potential measurements

The mitochondrial ΔΨ was measured simultaneously with oxygen uptake using a tetraphenylphosphonium (TPP^+)-specific electrode according to Kamo *et al.* [24]. Measurements were performed in the presence of 6 μM TPP^+. To calculate the ΔΨ value, the matrix volume of potato tuber mitochondria was assumed to be 2.0 μl × mg^{-1} protein. The calculation assumes that the TPP^+ distribution between mitochondria and medium followed the Nernst equation. Corrections were made for TPP^+ binding to mitochondrial membranes. Values of ΔΨ are presented in mV.

SDS-PAGE and immunoblotting

Samples of isolated mitochondrial proteins, SMP or the plasma membrane-enriched fraction were solubilized in sample buffer containing 2% (w/v) SDS, 50 mM Tris/Cl (pH.6.8), 10% glycerol, 0.004% (w/v) bromophenol blue, and 8% mercaptoethanol, and then boiled for 4 min. Proteins were separated in 12.5% SDS-polyacrylamide gels, and then electrotransferred to a nitrocellulose membrane. Membranes were then hybridized with anti-K_{Ca}1.1 and anti-*slo*β2 antibodies (APC-107 and APC-034, Alomone) at dilutions of 1:500 or 1:200, respectively, in the presence or absence of blocking peptide. Cross reactivity was also checked with antibodies raised against a plant plasma membrane H^+-ATPase (at a dilution of 1:1000). Protein detection was achieved with secondary antibodies linked to horseradish peroxidase (at a dilution of 1:20000) and the ECL chemiluminescence system. Protein content was determined by the Bradford method (Bio-Rad).

RESULTS

Effect of potassium channel modulators on respiratory rate and membrane potential in isolated potato tuber mitochondria

To investigate the possible effects of Ca^{2+} on the potassium permeability of isolated potato tuber mitochondria, we measured mitochondrial resting (nonphosphorylating) respiration and ΔΨ in potassium-containing medium in the presence of different Ca^{2+} concentrations, using succinate as an oxidizable substrate (Figure 1). An example of simultaneous measurements of the respiratory rate and ΔΨ is shown in Figure 1A. Addition of Ca^{2+} up to a concentration of 1 mM caused an increased respiration rate of up to 20 ± 2% in the absence of 1.5 μM IbTx, and up to 5 ± 3% in its presence (n = 4, S.D.) (Figure 1C). At the same time, ΔΨ decreased after addition of Ca^{2+} by up to 6.8 ± 0.5 mV and 0.7 ± 0.4 mV in the absence and presence of IbTx, respectively (n = 4, S.D.) (Figure 1D). Thus, the inhibitor previously described as specific for the mammalian large-conductance potassium channel [1,2] considerably blocks Ca^{2+}-induced respiration and ΔΨ depolarization in isolated potato tuber mitochondria. These results suggest that Ca^{2+} stimulates IbTx-sensitive K^+ flux into potato tuber mitochondria, decreasing ΔΨ and thus accelerating the mitochondrial respiration rate. This indicates that the mitoBK_{Ca} channel is present in potato tuber mitochondria. The Ca^{2+}-induced IbTx-sensitive respiratory rate (*i.e.*, the difference between the respiratory rate in the presence of Ca^{2+} and the rate in the presence of Ca^{2+} plus inhibitor) should represent mitoBK_{Ca} channel activity (*i.e.*, the channel-mediated K^+ flux). This activity, as measured using various Ca^{2+} concentrations (Figure 1B), revealed that 50% of the maximal stimulation ($S_{0.5}$) by Ca^{2+} is reached at 0.45 mM, as calculated from the linear regression of a double reciprocal plot (Figure 1B, *inset*). A similar value was attained when the Ca^{2+}-induced IbTx-sensitive depolarization of ΔΨ versus the Ca^{2+} concentration was analyzed (data not shown). The partial insensitivity of Ca^{2+}-induced respiration and ΔΨ depolarization to IbTx observed in potato tuber mitochondria is likely related to electrophoretic Ca^{2+} influx pathways present in some plant mitochondria [25] or permeability transition opening [26].

The potassium channel opener NS1619, previously described to be specific for the mitoBK_{Ca} channel [1,2], was also used in our studies. Similarly to calcium ions, NS1619 stimulates mitochondrial resting oxygen uptake and decreases mitochondrial resting ΔΨ (Figure 2). An example of the effect of increasing concentrations of NS1619 on respiratory rate and ΔΨ is shown in Figure 2A. Addition of NS1619 up to 40 μM resulted in an increase in the rate of respiration up to 100 ± 6% and 53 ± 7% (n = 6, S.D.) in the

Licenced copy. Copying is not permitted, except with prior permission and as allowed by law.
© 2009 The Authors Journal compilation © 2009 Portland Press Limited

absence and presence of 1.5 μM IbTx, respectively (Figure 2C). At the same time, ΔΨ decreased after addition of up to 40 μM NS1618 by up to 18.1 ± 2 mV and 10.6 ± 1.3 mV (n = 6, S.D.) in the absence and presence of IbTx, respectively (Figure 2D). Thus, IbTx partially blocks (approximately 50%) NS1619-induced respiration and ΔΨ depolarization in isolated potato tuber mitochondria. When compared to the effect of IbTx on Ca^{2+}-induced respiration and ΔΨ depolarization (Figure 3), this could indicate a non-specific uncoupling effect of NS1619 on isolated potato tuber mitochondria rather than low sensitivity of the channel to IbTx. Similarly, the sensitivity of NS1619-induced respiration and ΔΨ depolarization to the mitoBK_{Ca} channel blockers (IbTx, charybdotoxin, paxilline) in some mammalian isolated mitochondria is not complete [27-30]. Nevertheless, we can conclude that in potato tuber mitochondria, NS1619 stimulates IbTx-sensitive K^+ flux, decreases ΔΨ, and thus accelerates the mitochondrial respiration rate, further indicating the presence of the mitoBK_{Ca} channel activity. The NS1619-induced IbTx-sensitive respiratory rate (*i.e.*, the difference between the respiratory rate in the presence of the modulator and the rate after addition of the inhibitor) measured at various NS1619 concentrations revealed that 50% of maximal stimulation ($S_{0.5}$) is reached at 25 μM NS1619 [Figure 2B, *inset*]. When NS1619-induced IbTx-sensitive depolarization of ΔΨ at various NS1619 concentrations was plotted, a similar value of $S_{0.5}$ for NS1619 was obtained (data not shown).

Figure 3 illustrates the efficiency of IbTx in the inhibition of mitoBK_{Ca} channel activity under unstimulated (no activators) or stimulated (in the presence of 0.8 mM $CaCl_2$ or 40 μM NS1619) conditions. An example of the effect of increasing concentrations of IbTx on NS1619-induced respiration and ΔΨ depolarization is shown in Figure 3A. Under all studied conditions, addition of IbTx up to a concentration of 1.5 μM blocked unstimulated, NS1619-induced, and Ca^{2+}-induced respiration by up to 4%, 53%, and 79%, respectively (Figure 3C). Similarly, IbTx restored up to 1%, 46% and 78% of unstimulated, NS1619-induced and Ca^{2+}-induced ΔΨ depolarization, respectively (Figure 3D). These results indicate low activity of the mitoBK_{Ca} channel in the absence of modulators in isolated potato tuber mitochondria, as IbTx has a slight inhibitory effect. Moreover, the results reveal that IbTx inhibits Ca^{2+}-induced mitoBK_{Ca} channel activity (~80% inhibition) more efficiently than NS1619-induced channel activity (~50% inhibition) again indicating a non-specific uncoupling effect of NS1619 on isolated potato tuber mitochondria. The relationship between NS1619-induced IbTx-sensitive respiration and various IbTx concentrations reveals that 50% of maximal inhibition ($I_{0.5}$) by IbTx is reached at 615 nM, as calculated from the linear regression of a double reciprocal plot (Figure 3B, *inset*). A similar value was obtained when the effect of increasing IbTx concentration on ΔΨ depolarization was analyzed (data not shown).

To exclude an influence of the applied modulators (Ca^{2+}, Ns1619, IbTx) on succinate dehydrogenase activity, experiments were also performed with external NADH as a respiratory substrate (data not shown). The results confirm observations taken from the experiments described above (Figures 1-3).

The findings presented herein strongly suggest the presence of the Ca^{2+}-activated potassium channel in the inner membrane of potato tuber mitochondria. To further test this hypothesis, we investigated the effect of Ca^{2+} and IbTx on isolated potato tuber mitochondria respiring (with succinate) in incubation media containing different monovalent cations (chloride salts) compared to those incubated in the presence of K^+ (Figure 4) or in the absence of any monovalent cations. In medium deprived of monovalent cations, no Ca^{2+}-induced IbTx-sensitive increase in respiration and ΔΨ depolarization were observed (data not shown). Figure 4 shows the influence of increasing concentrations (up to 50 mM) of KCl, NaCl, LiCl, RbCl, and CsCl on Ca^{2+}-induced IbTx-sensitive ΔΨ depolarization (*i.e.*, the difference between ΔΨ in the presence of 0.8 mM $CaCl_2$ and ΔΨ in the presence of $CaCl_2$ and 1.5 μM IbTx). The results indicate that the influence of potassium channel modulators (Ca^{2+} and IbTx) on isolated potato tuber mitochondria in the above experiments can be significantly attributed to K^+ influx through the inner membrane.

Electrophysiological properties of mitoBK_{Ca} channel from the potato tuber mitochondrial membrane

The inner mitochondrial membrane-enriched SMP from potato tuber mitochondria were reconstituted into PLMs. Incorporation of channels into the bilayer was usually observed within 10-20 min after SMP addition to the *trans* compartment. SMP fractions from five different preparations were used. More than

THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/BJ20090991

Accepted Manuscript

Licenced copy. Copying is not permitted, except with prior permission and as allowed by law.
© 2009 The Authors Journal compilation © 2009 Portland Press Limited

140 reconstitutions were monitored in the single-channel experiments. Approximately 40% of the channels incorporated into bilayers showed properties of the large-conductance potassium channels. Figure 5 shows representative current-time traces (Figure 5B) and current-voltage relationship (Figure 5C) for potassium channel opening at different voltages in the 50/450 mM KCl (*cis*/*trans*) gradient solutions. The current was measured as a function of applied potential at intervals ranging from +90 mV to -30 mV (Figure 5C). The calculated ion conductance is equal to 502 ± 15 pS for potential from -30 to 20 mV and 615 ± 12 pS for potential from +40 to 90 mV, thus indicating large-conductance potassium channel activity. The reversal potential of +34 mV calculated from curve fitting to the experimental data indicates that the examined ion channel is cation-selective. All incorporated large-conductance potassium channels were more active at positive than at 0 mV potentials (Figure 5D). Sometimes we recorded spontaneous closing of channels at 0 mV and at negative potentials (Figure 5B). The activity of such channels could be rescued after applying positive potentials, for example 50 mV.

Substances known to modulate mammalian mitoBK_{Ca} channel activity were used to examine the properties of the K^+ ion channel observed in our experiments. Figure 6 demonstrates the effect of 300 µM Ca^{2+} on the potato tuber mitochondrial large-conductance potassium channels. Figure 6A shows single channel recordings in the 50/450 mM KCl (*cis*/*trans*) gradient solutions at 0 mV, before and after addition of Ca^{2+}. Ca^{2+} caused a transition into an open state that indicates the existence of the mitoBK_{Ca} channel in the potato tuber inner mitochondrial membrane. As depicted in the histograms shown in Figure 6B, the number of observations of the channel being in an open state changed from 73% to 86%. The probability of channel opening (P) at 0 mV potential increased from 0.49 ± 0.07 to 0.84 ± 0.05 (n = 4, $p<0.0076$, unpaired t test) in the presence of Ca^{2+} (Figure 6C).

Figure 7 presents the inhibitory effect of IbTx on the potato tuber mitochondrial large-conductance potassium channels. As shown in the representative single channel recording obtained in 50/450 mM KCl (*cis*/*trans*) gradient solutions at 0 mV, before and after the addition of 600 nM IbTx, the inhibitor caused a complete transition of the channel protein into a closed state (Figure 7A). The number of open state events changed from 83% to zero after IbTx addition, as illustrated in the histograms (Figure 7B). The probability of channel opening (P) at 0 mV potential decreased from 0.75 ± 0.27 for control recordings to 0.049 ± 0.04 after addition of 600 nM IbTx ($p<0.008$, unpaired t test). Moreover, IbTx blocked the channel activity in a dose-dependent manner (in a range from 200 to 600 nM) (Figure 7C). The apparent concentration of IbTx that provided half maximal inhibition ($I_{0.5}$) was approximately 170 nM. In our PLM experiments, 25% of the channels with a large conductance did not display sensitivity to IbTx (data not shown).

Immunological detection of plant mitoBK_{Ca} channel proteins

Immunoblotting of total mitochondrial proteins, as well as SMP, allowed immunological detection of the plant mitoBK_{Ca} channel. For this, antibodies raised against the mammalian plasma membrane BK_{Ca} channel pore (α subunit $K_{Ca}1.1$) were used. In plant mitochondrial and SMP fractions, a protein band with a molecular mass of approximately 70 kDa was detected using the anti-$K_{Ca}1.1$ antibodies (Figure 8A). We also used antibodies raised against the mammalian plasma membrane BK_{Ca} channel β subunits. In potato tuber mitochondrial and SMP fractions, anti-*slo*β2 antibodies cross-reacted with a single band at around 30 kDa (Figure 8B). Less sensitive reactivity was observed with anti-*slo*β1 and anti-*slo*β4 antibodies (data not shown). As shown in Figure 8, much stronger signals were obtained with SMP compared to isolated mitochondria using anti-$K_{Ca}1.1$ and anti-*slo*β2 antibodies, proving that the detected proteins localized to the inner membrane of potato tuber mitochondria. Moreover, specific blocking peptides blocked the antibody-antigen interaction, demonstrating the specificity of the reaction in the western blot analysis. On the other hand, mitochondrial (isolated mitochondria and SMP) and plasma membranes probed with antibodies to a plant plasma membrane marker (H^+-ATPase) displayed no signal in the mitochondrial fractions, indicating the absence of surface membrane contamination (Figure 8C). Therefore, we can conclude that the potato tuber mitoBK_{Ca} channel may contain subunits similar to the α subunit $K_{Ca}1.1$ and the β subunit *slo*β2.

THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/BJ20090991

Accepted Manuscript

Licenced copy. Copying is not permitted, except with prior permission and as allowed by law.
© 2009 The Authors Journal compilation © 2009 Portland Press Limited

THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/BJ20090991

Accepted Manuscript

DISCUSSION

In the present study, we describe for the first time (to our knowledge) the functional properties of the mitoBK_{Ca} channel-like protein in plant mitochondria. Potassium channel activators, Ca^{2+} and NS1619, are able to modulate the resting respiratory rate (stimulation) and $\Delta\Psi$ (depolarization) in isolated potato tuber mitochondria. The opposite responses (respiratory rate inhibition and $\Delta\Psi$ repolarization) were observed when the mitoBK_{Ca} channel inhibitor, IbTx, was applied. These effects were dependent on the presence of K^+ in the incubation medium. Thus, we provide evidence that the observed effects of the mitoBK_{Ca} channel openers are due to activation of electrogenic potassium transport through the inner mitochondrial membrane, likely mediated by a potassium channel belonging to the family of mitochondrial potassium channels previously described in mammalian mitochondria [7,12].

This is the first study to investigate the electrophysiological properties of the large-conductance potassium channels in plant mitochondria. We used the planar lipid bilayers technique to confirm that large potassium channels are present in the potato tuber inner mitochondrial membrane. The cation selectivity, large conductance (502-615 pS), reverse potential (+34 mV) and sensitivity to Ca^{2+} and IbTx of the potato tuber mitoBK_{Ca} channel indicate similarity to the mammalian mitoBK_{Ca} channels previously reported in glioma [8], skeletal muscle [29], brain [30], and cardiac [31] mitochondria. However, the ion conductance of the potato tuber mitoBK_{Ca} channel (502-615 pS) is higher than that observed for mammalian mitoBK_{Ca} channels (~260-300 pS). This could be due to highly active K^+ transport in some plant mitochondria, leading to enormous mitochondrial depolarization (also in potato tuber mitochondria) [14], which contrasts with mammalian mitochondria in which $\Delta\Psi$ dissipation seems to be weaker [32,33]. Moreover, mammalian plasma membrane BK_{Ca} channel has a higher relative permeability for Rb^+ than for Na^+ [34] in contrast to that observed for potato tuber mitoBK_{Ca} channel. However, K^+ flux is dominant.

In addition to the functional studies, the presence of mitoBK_{Ca} channel proteins in potato tuber mitochondria is indicated by their cross-reactivity with antibodies raised against the $K_{Ca}1.1$ α subunit of the mouse BK_{Ca} channel and the β subunit of the human sloβ2 BK_{Ca} channel. This suggests that the mitoBK_{Ca} channel present in the potato tuber inner mitochondrial membrane may be structurally similar to the mammalian BK_{Ca} channel. Namely, it may be formed by the principle pore-forming α subunit that interacts with an auxiliary β2 subunit. The predominant β2 subunit may determine the channel's activity, including its sensitivity to Ca^{2+} and other modulators. The relative molecular mass of the detected potato tuber proteins (around 70 and 30 kDa for the α and β2 subunits, respectively) differs from that of the mammalian proteins from plasma membrane (~125 and ~44 kDa for the α and β2 subunits, respectively) [35], while the molecular mass of the α subunit in mammalian mitochondria is variable, depending on tissue (55-125 kDa) [31,36]. The β2 subunit of mitoBK_{Ca} was detected in astrocyte mitochondria [37]. However, the molecular identity (gene and protein sequences) of the mammalian mitoBK_{Ca} channel is presently unknown. On the other hand, the molecular mass of the detected potato tuber mitoBK_{Ca} channel α subunit (~70 kDa) is similar to the plant potassium channel protein from the plasma membrane of *Arabidopsis thaliana* (76 kDa) [38].

Our results demonstrate the presence of a mitoBK_{Ca}-channel in plant mitochondria that may function as a possible novel signaling link between intramitochondrial calcium levels and the mitochondrial membrane potential. Ion channels in the mitochondrial inner membrane influence cell function in specific ways that can be detrimental or beneficial to plant cell function. At least one type of potassium channel, the mitoK_{ATP} channel, has been described in plant mitochondria to date [14-17]. The physiological functions of the plant mitoK_{ATP} channel remain unclear. It may be involved in regulating mitochondrial volume [15], in programmed cell death (19), and/or in the prevention of reactive oxygen species formation [14,20]. The physiological role of the mitoBK_{Ca} channel in the plant mitochondria described in this work, which seems to significantly modulate K^+ mitochondrial distribution, awaits exploration.

In summary, in the present study we identified and characterized a novel potassium channel of the inner membrane of potato tuber mitochondria. Pharmacological, biophysical, and molecular properties of the potato tuber mitochondrial potassium channel are similar to those of the well known mammalian plasma membrane BK_{Ca}, including: (i) potassium selectivity and activation by calcium ions and NS1619 (a BK_{Ca}

Licenced copy. Copying is not permitted, except with prior permission and as allowed by law.
© 2009 The Authors Journal compilation © 2009 Portland Press Limited

opener); (ii) inhibition by IbTx (a BK_{Ca} blocker); (iii) biophysical properties of a large-conductance potassium channel; (iv) immunoreactivity with antibodies raised against α and β subunits of the plasma membrane BK_{Ca}. These properties strongly suggest that a large-conductance calcium-activated potassium channel similar to that of mammalian mitochondria is present in plant mitochondria. The functional and immunoblotting data presented herein demonstrate for the first time the presence of a mitoBK_{Ca} channel in the inner membrane of plant mitochondria.

This work was supported by the Polish Mitochondrial Network MitoNet.pl and by the Nencki Institute of Experimental Biology. Partially the study was supported by grants from the Ministry of Science and Higher Education P-N/031/2006 and 3382/B/P01/2007/33.

REFERENCES

1. O'Rourke, B. (2004) Evidence for mitochondrial K+ channels and their role in cardioprotection. Circ. Res. **94**, 420-432
2. Szewczyk, A., Jarmuszkiewicz, W. and Kunz, W.S. (2009) Mitochondrial potassium channels. IUBMB Life **61**, 134-143
3. Szewczyk, A. (1998) The intracellular potassium and chloride channels: properties, pharmacology and function. Mol. Membr. Biol. **15**, 49-58
4. Bernardi, P. (1999) Mitochondrial transport of cations: channels, exchangers, and permeability transition. Physiol. Rev. **79**, 1127-1155
5. Szewczyk, A. and Wojtczak, L. (2002) Mitochondria as a pharmacological target. Pharmacol. Rev. **54**, 101-127
6. Halestrap, A. P. (1994) Regulation of mitochondrial metabolism through changes in matrix volume. Biochem. Soc. Trans. **22**, 522-529
7. Inoue, I., Nagase, H. Kishi, K. and Higuti, T. (1991) ATP-sensitive K^+ channel in the mitochondrial inner membrane. Nature **352**, 244-247
8. Siemen, D., Loupatatzis, C., Borecky, J. Gulbins, E. and Lang, F. (1999) Ca^{2+}-activated K channel of the BK-type in the inner mitochondrial membrane of a human glioma cell line. Biochem. Biophys. Res. Commun. **257**, 549-554
9. Szabo, I., Bock, J. Jekle, A. Soddemann, M. Adams, C. Lang, F. Zoratti, M. and Gulbins, E. (2005) A novel potassium channel in lymphocyte mitochondria. J. Biol. Chem. **280**, 12790-12798
10. Jarmuszkiewicz, W., Sluse-Goffart, CM., Vercesi, A.E. and Sluse, F.E. (2001) Alternative oxidase and uncoupling protein: thermogenesis versus cell energy balance. Biosci. Rep. **21**, 213-222
11. Sluse, F.E. and Jarmuszkiewicz, W. (1998) Alternative oxidase in the branched mitochondrial respiratory network: an overview on structure, function, regulation, and role. Braz. J. Med. Biol. Res. **31**, 733-747
12. Siedow, J.N. and Umbach, A.L. (2000) The mitochondrial cyanide-resistant oxidase: structural conservation amid regulatory diversity. Biochim. Biophys. Acta **1459**, 432-439
13. Vercesi, A.E., Borecky, J., Maia, I.D., Arruda, P., Cuccovia, I,M. and Chaimovich, H. (2006) Plant uncoupling mitochondrial proteins. Annu. Rev. Plant Biol. **57**, 383-404
14. Pastore, D., Stoppelli, M. C., Di Fronzo, N. and Passarella, S. (1999) The existence of the K^+ channel in plant mitochondria. J. Biol. Chem. **274**, 26683-26690
15. Petrussa, E., Casolo, V., Braidot, E., Chiandussi, E., Macri, F. and Vianello, A. (2001) Cyclosporin A induces the opening of a potassium-selective channel in higher plant mitochondria. J. Bioenerg. Biomembr. **33**, 107–117
16. Chiandussi, E., Petrussa, E., Macri, F. and Vianello, A. (2002) Modulation of a plant mitochondrial K^+_{ATP} channel and its involvement in cytochrome *c* release. J. Bioenerg. Biomembr. **34**, 177–184
17. Diolez, P. and Moreau, F. (1985) Correlations between ATP-synthesis, membrane potential and oxidation rate in plant mitochondria. Biochim. Biophys. Acta **806**, 56–63

THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/BJ20090991

Accepted Manuscript

Licenced copy. Copying is not permitted, except with prior permission and as allowed by law.
© 2009 The Authors Journal compilation © 2009 Portland Press Limited

18. Pastore, D., Trono, D., Laus, M. N., Di Fronzo, N. and Flagella, Z. (2007) Possible plant mitochondria involvement in cell adaptation to drought stress. A case study: durum wheat mitochondria. J. Exp. Bot. **58**, 195-210.
19. Casolo, V., Petrussa, E., Krajnáková, J., Macrì, F. and Vianello, A. (2005) J. Exp. Bot. Involvement of the mitochondrial K^+_{ATP} channel in H_2O_2- or NO-induced programmed death of soybean suspension cell cultures. **56**, 997-1006
20. Ruy, F., Vercesi, A.E., Andrade, P.B., Bianconi, M.L., Chaimovich, H. and Kowaltowski, A.J. (2004) A Highly active ATP-insensitive K+ import pathway in plant mitochondria. J. Bioenerg. Biomembr. **36**, 195-202
21. Bednarczyk, P., Kicinska, A., Kominkova, V., Ondrias, K., Dołowy K. and Szewczyk, A. (2004) Quinine inhibits mitochondrial ATP-regulated potassium channel from bovine heart. J. Membr. Biol. **199**, 63-72
22. Hordejuk, R., Lobanov, N. A., Kicinska, A., Szewczyk, A. and Dolowy, K. (2004) pH modulation of large conductance potassium channel from adrenal chromaffin granules. Mol. Membr. Biol. **21**, 307-313
23. Bednarczyk, P., Dołowy, K. and Szewczyk, A. (2005) Matrix Mg^{2+} regulates mitochondrial ATP-dependent potassium channel from heart. FEBS Lett. **579**, 1625-1632
24. Kamo, N., Muratsugu, M., Hongoh, R. and Kobatake, Y. (1979) Membrane potential of mitochondria measured with an electrode sensitive to tetraphenyl phosphonium and relationship between proton electrochemical potential and phosphorylation potential in steady state. J. Membr. Biol. **49**, 105-121
25. Silva, M.A.P., Carnieri, E.G.S. and Vercesi, A.E. (1992) Calcium transport by corn mitochondria: evaluation of the role of phosphate. Plant Physiol. **98**, 452-457
26. de Oliveira, H.C., Saviani, E.E., de Oliveira, J.F.P. and Salgado, I. (2007) Cyclosporin A inhibits calcium uptake by *Citrus sinensis* mitochondria. Plant Sci. **172**, 665-670
27. Heinen, A., Camara, A.K.S., Aldakkak, M., Rhodes, S.S., Riess, M.L. and Stowe, D.F. (2006) Mitochondrial Ca^{2+}-induced K^+ influx increases respiration and enhances ROS production while maintaining membrane potential. Am. J. Physiol. Cell Physiol. **292**, C148-C156
28. Heinen, A., Winning, A., Shlack, W., Hellmann, M.W., Precel, B., Fraβdorf, J. and Weber, N.C. (2008) Physiological levels of glutamine prevent morphine-induced preconditioning in the isolated rat heart. Eur. J. Pharmacol. **578**, 108-113
29. Skalska, J., Piwonska, M., Wyroba, E., Surmacz, L., Wieczorek, R., Koszela-Piotrowska, I., Zielinska, J., Bednarczyk, P., Dolowy, K., Wilczyński, G. M., Szewczyk, A. and Kunz, W. S. (2008) A novel potassium channel in skeletal muscle mitochondria. Biochim. Biophys. Acta **1777**, 651-659
30. Skalska, J., Bednarczyk, P., Piwonska, M., Kulawiak, B., Wilczynski, G., Dolowy, K., Kudin, A. P., Kunz, W.S. and Szewczyk, A. (2009) Calcium ions regulate K uptake into brain mitochondria: the evidence for a novel potassium channel. Int. J. Mol. Sci. **10**, 1104-1120
31. Xu ,W., Liu, Y., Wang, S., McDonald, T., Van Eyk, J.E., Sidor, A. and O'Rourke, B. (2002) Cytoprotective role of Ca^{2+}- activated K^+ channels in the cardiac inner mitochondrial membrane. Science **298**, 1029-1033
32. Debska, G., Kicinska, A., Skalska, J., Szewczyk, A., May, R., Elger, C.E. and Kunz, W.S. (2002) Opening of potassium channels modulates mitochondrial function in rat skeletal muscle. Biochim Biophys Acta **1556,** 97-105
33. Debska, G., May, R., Kicińska, A., Szewczyk, A., Elger, C.E. and Kunz, W.S. (2001) Potassium channel openers depolarize hippocampal mitochondria. Brain Res, **892,** 42-50
34. Blatz, A.L. and Magleby, K.L. (1984) Ion conductance and selectivity of single calcium-activated potassium channels in cultured rat muscle. J. Gen. Physiol. **84**, 1-23
35. Wulf, H., Hay-Schmidt, A., Poulsen, A.N., Klaerke, D.A., Olesen, J. and Jansen-Olesen, I. (2009) Molecular investigations of BK(Ca) channels and the modulatory beta-subunits in porcine basilar and middle cerebral arteries. J. Mol. Histol. **40**, 87-97

THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/BJ20090991

Accepted Manuscript

Licenced copy. Copying is not permitted, except with prior permission and as allowed by law.
© 2009 The Authors Journal compilation © 2009 Portland Press Limited

36. Douglas, R.M., Lai, J.C.K., Bian, S., Cummins, L., Moczydlowski, E. and Haddad, G.G. (2006) The calcium-sensitive large-conductance potassium channel (BK/MAXI K) is present in the inner mitochondrial membrane of rat brain. Neuroscience **139**, 1249-1261
37. Piwonska, M., Wilczek, E., Szewczyk, A. and Wilczyński, G.M. (2008) Diferential distribution of Ca^{2+}-activated channel β4 subunit in rat brain: immunolocalization in neuronal mitochondria. Neuroscience **153,** 446-60
38. Reintanz, B., Szyroki, A., Ivashikina, N., Ache, P., Godde, M., Becker, D., Palme, K. and Hedrich, R. (2002) AtKC1, a silent Arabidopsis potassium channel alpha -subunit modulates root hair K+ influx. Proc. Natl. Acad. Sci. USA **99**, 4079-4084

FIGURE LEGENDS

Figure 1 Iberiotoxin-sensitive influence of $CaCl_2$ on resting respiratory rate and membrane potential.
Mitochondria were incubated in a standard incubation medium additionally containing 50 mM KCl. *A*, 5 mM succinate, 1.5 μM IbTx and successive doses of $CaCl_2$ were added as indicated. The dashed trace shows the measurement obtained in the absence of Ca^{2+}. An example of three measurements (using mitochondria from three different preparations) is shown. Numbers on the traces refer to O_2 consumption rates in nmol O × min^{-1} × mg^{-1} protein or to ΔΨ values in mV. *B*, Ca^{2+}-induced IbTx-sensitive respiration versus $CaCl_2$ concentration. *Inset*, double reciprocal plot. *C*, *D*, effect of $CaCl_2$ concentration on O_2 consumption and ΔΨ (respectively) in the presence or absence of 1.5 μM IbTx. Course of the measurements as in *A*, except that IbTx was added prior to addition of Ca^{2+} where indicated. The data deal with three different mitochondrial preparations (± S.D). *C*, the percentage of control respiration in the absence of modulators (119 ± 4 nmol O × min^{-1} × mg^{-1} protein). *D*, the change in ΔΨ induced by a given concentration of $CaCl_2$ relative to the initial state 4 ΔΨ (216 ± 1 mV, S.D.) in the absence of modulators.

Figure 2 Influence of NS1619, a mitoBK_{Ca} channel activator, on resting respiratory rate and membrane potential. Mitochondria were incubated in a standard incubation medium additionally containing 50 mM KCl. *A*, 5 mM succinate, 1.5 μM IbTx, and successive doses of NS1619 were added as indicated. The dashed trace shows the measurement obtained in the absence of activator. Numbers on the traces refer to O_2 consumption rates in nmol O × min^{-1} × mg^{-1} protein or to ΔΨ values in mV. *B*, NS1619-induced IbTx-sensitive respiration versus NS1619 concentration. *Inset*, double reciprocal plot. *C*, *D*, effect of NS1619 concentration on O_2 consumption and ΔΨ (respectively) in the presence or absence of 1.5 μM IbTx. Course of the measurements as in *A*, except that IbTx was added prior to activator addition where indicated. The data deal with three different mitochondrial respirations (± S.D). *C*, the percentage of control respiration in the absence of modulators (122 ± 3 nmol O × min^{-1} × mg^{-1} protein). *D*, the change in ΔΨ induced by a given concentration of $CaCl_2$ relative to the initial state 4 ΔΨ (217 ± 1 mV, S.D.) in the absence of modulators.

Figure 3 Influence of iberiotoxin, a mitoBK_{Ca} channel inhibitor, on resting respiratory rate and membrane potential. Mitochondria were incubated in a standard incubation medium additionally containing 50 mM KCl. *A*, 5 mM succinate, 40 μM NS1619, and successive doses of 0.5 μM IbTx were added as indicated. The dashed trace shows the measurement in the absence of IbTx addition. Numbers on the traces refer to O_2 consumption rates in nmol O × min^{-1} × mg^{-1} protein or to ΔΨ values in mV. *B*, IbTx-sensitive NS1619-induced respiration versus the inhibitor concentration. *Inset*, double reciprocal plot. *C*, *D*, effect of IbTx concentration on O_2 consumption and ΔΨ (respectively) in the presence or absence of 0.8 mM $CaCl_2$ or 40 μM NS1619. Course of measurements as in *A*. The data deal with three different mitochondrial respirations (± S.D). *C*, the percentage of control respiration in the absence of activators and IbTx (120 ± 4 nmol O × min^{-1} × mg^{-1} protein). *D*, the change in ΔΨ induced by a given modulator relative to the initial state 4 ΔΨ (216 ± 1 mV, S.D.).

THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/BJ20090991

Accepted Manuscript

Licenced copy. Copying is not permitted, except with prior permission and as allowed by law.
© 2009 The Authors Journal compilation © 2009 Portland Press Limited

THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/BJ20090991

Figure 4 Cation selectivity; influence of cations on Ca^{2+}-induced IbTx-sensitive resting respiratory rate and membrane potential. Mitochondria were incubated in a standard incubation medium, except that 3 mM NaH_2PO_4 was replaced with 3 mM $TrisH_2PO_4$. Increasing concentrations of KCl, NaCl, LiCl, CsCl, or RbCl (10-50 mM) were obtained by successive additions once a steady-state ΔΨ had been established. The dilution effect of salt addition was taken into account. The change in ΔΨ induced by a given chloride salt relative to the initial state 4 ΔΨ (216 ± 1 mV, S.D.) is shown as the difference between ΔΨ measured in the presence of 0.8 mM $CaCl_2$ and ΔΨ measured in the presence of $CaCl_2$ and 1.5 µM IbTx.

Figure 5 Single channel recordings of large-conductance potassium channels from potato tuber mitochondria in planar lipid bilayers. *A*, Schematic configuration of the *cis* and *trans* compartments used in the experiments. Reconstitution of inner mitochondrial membranes into planar lipid bilayers was performed as described under "Experimental Procedures". *B*, Single channel current-time recordings in 50/450 mM KCl (*cis/trans*) gradient solutions at different voltages. "-" indicates current during the closed state of the channel. An example of each of eight measurements is shown. *C*, current-voltage (I/V) characteristics of single-channel events in 50/450 mM KCl (*cis/trans*) gradient solutions (n = 12) at different voltages. *D*, probability of channel opening (P) at two different voltages, 0 and 50 mV (mean ± SEM, n = 3).

Figure 6 Effects of Ca^{2+} on the activity of large-conductance potassium channels from potato tuber mitochondria. *A*, an example of the single channel recordings in 50/450 mM KCl (*cis/trans*) gradient solutions at 0 mV under control conditions and after addition of 300 µM Ca^{2+} to the *cis* and *trans* compartments (n = 4). "-" indicates current during the closed state of the channel. *B*, amplitude histograms measured in the absence and presence of 300 µM Ca^{2+}. All points shown in the amplitude histograms were fitted by two Gaussian distributions. The closed state corresponds to the peak at 0 pA, O - open state, C – closed state. *C*, Probability of channel opening (P) at 0 mV potential. The average of four different experiments (± SEM) is shown.

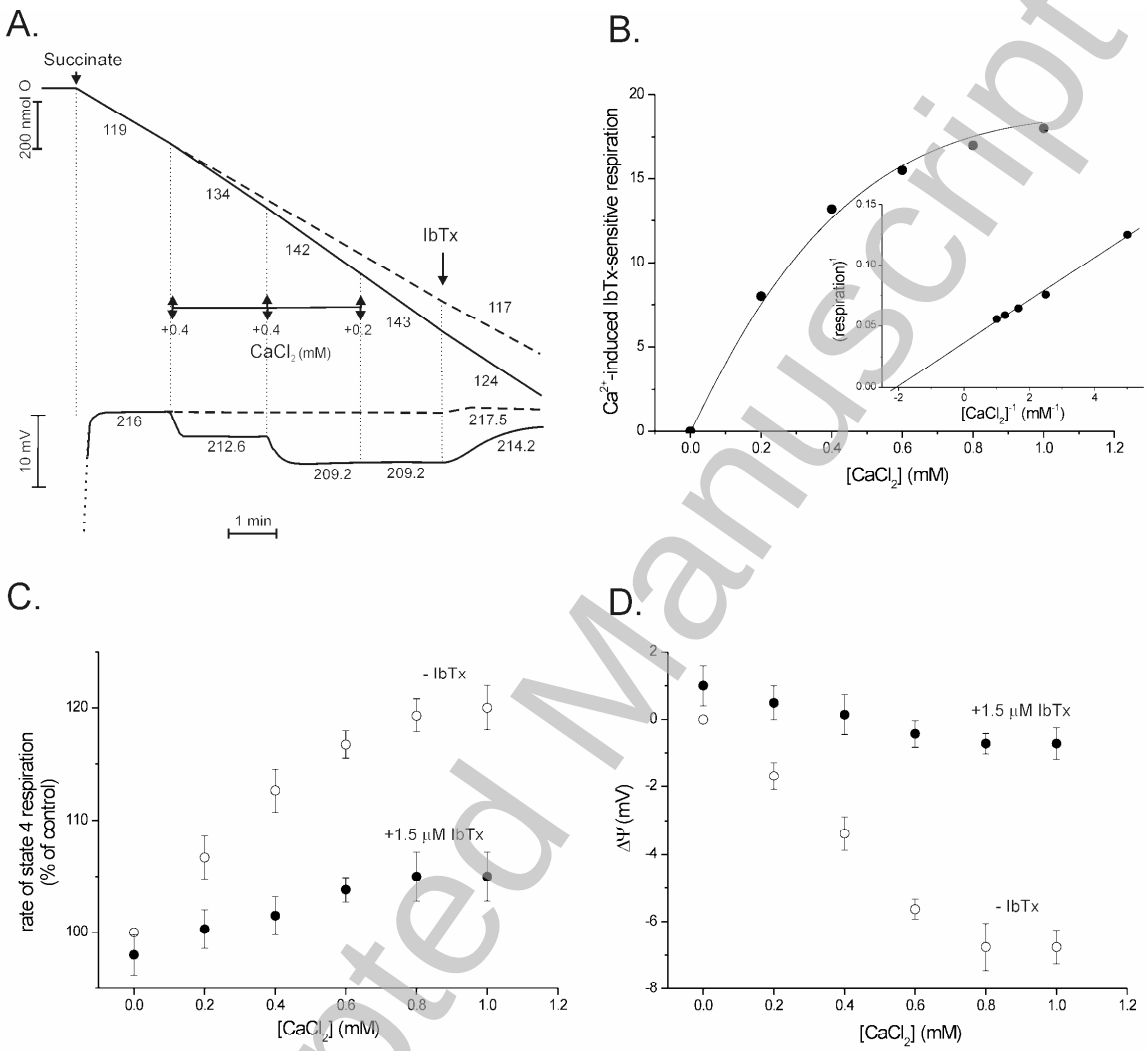
Figure 7 Effects of IbTx on the activity of large-conductance potassium channels from potato tuber mitochondria. *A*, an example of single channel recordings in 50/450 mM KCl (*cis/trans*) gradient solutions at 0 mV under control conditions and after addition of 600 nM IbTx to the *cis* and *trans* compartments. "-" indicates current during the closed state of the channel. *B*, amplitude histograms from a control experiment and after addition of 600 nM IbTx. All points shown in the amplitude histograms were fitted by two Gaussian distributions. The closed state corresponds to the peak at 0 pA, O – open state, C – closed state. *C*, Probability of channel opening (P) at 0 mV potential. Data from eight experiments in which different concentrations of IbTx were used are presented as means ± SEM.

Figure 8 Western blot analysis of potato tuber fractions with anti-K_{Ca}1.1 (*A*) and anti-*slo*β2 (*B*) antibodies raised against the mammalian BK_{Ca} channel proteins in the absence or presence of a specific blocking peptide. *C*, Detection of H^+-ATPase. Mito - mitochondria, SMP - submitochondrial particles, PM – plasma membrane-enriched fraction. Different amounts of protein were loaded into each lane (as indicated). Examples of 3-4 immunoblots (using samples from different preparations) are shown.

Accepted Manuscript

Licenced copy. Copying is not permitted, except with prior permission and as allowed by law.
© 2009 The Authors Journal compilation © 2009 Portland Press Limited

Fig. 1.

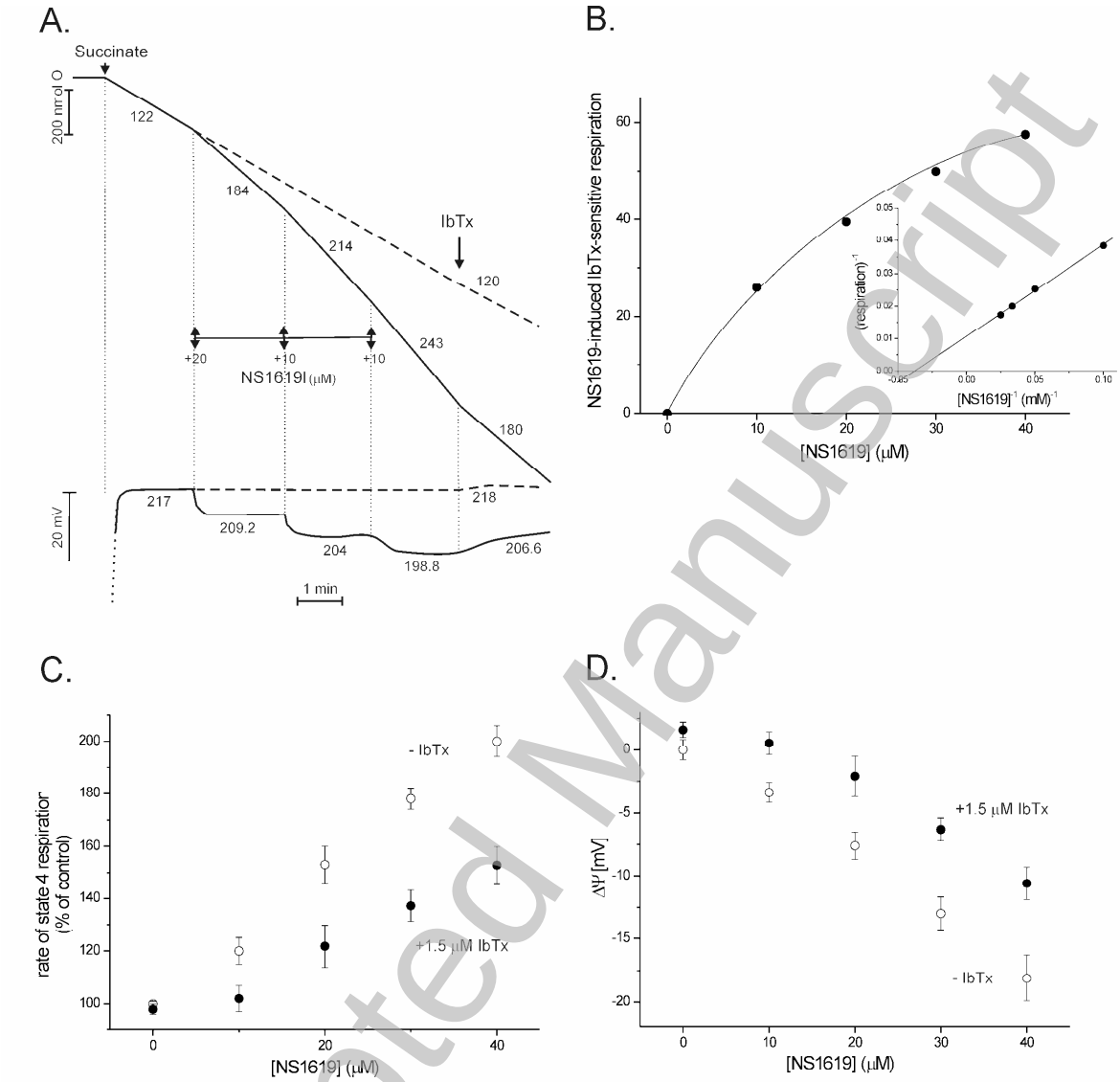


THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/BJ20090991

Accepted Manuscript

Licenced copy. Copying is not permitted, except with prior permission and as allowed by law.
© 2009 The Authors Journal compilation © 2009 Portland Press Limited

Fig. 2.

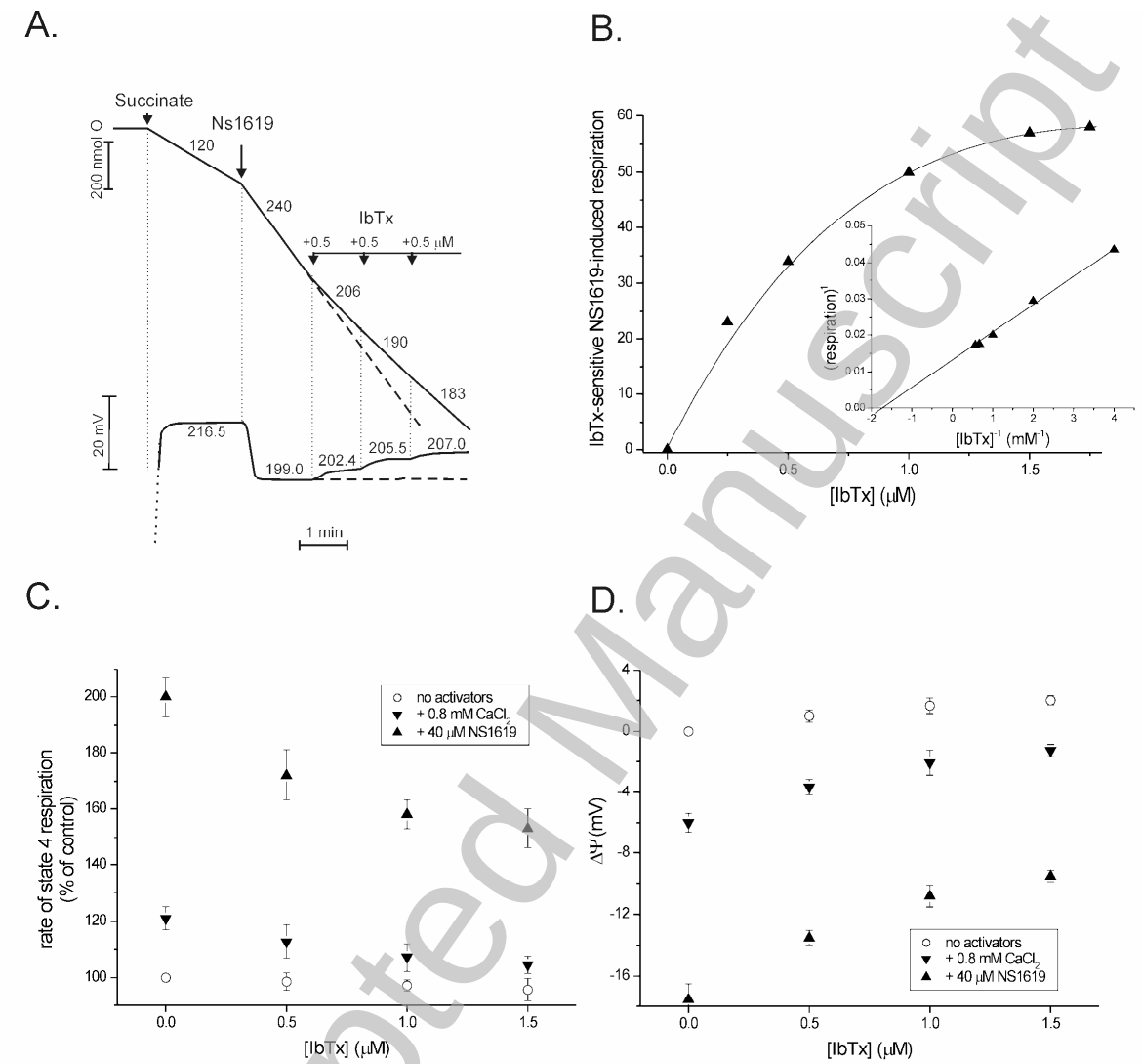


THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/BJ20090991

Accepted Manuscript

Licenced copy. Copying is not permitted, except with prior permission and as allowed by law.
© 2009 The Authors Journal compilation © 2009 Portland Press Limited

Fig. 3.

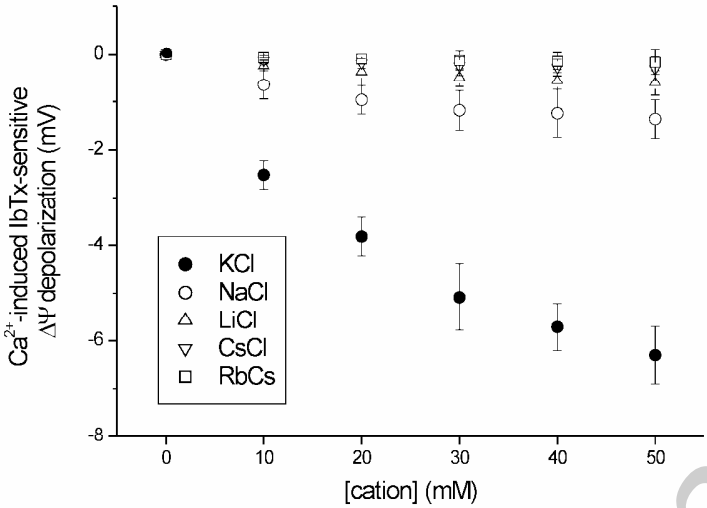


THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/BJ20090991

Accepted Manuscript

Licenced copy. Copying is not permitted, except with prior permission and as allowed by law.
© 2009 The Authors Journal compilation © 2009 Portland Press Limited

Fig. 4.

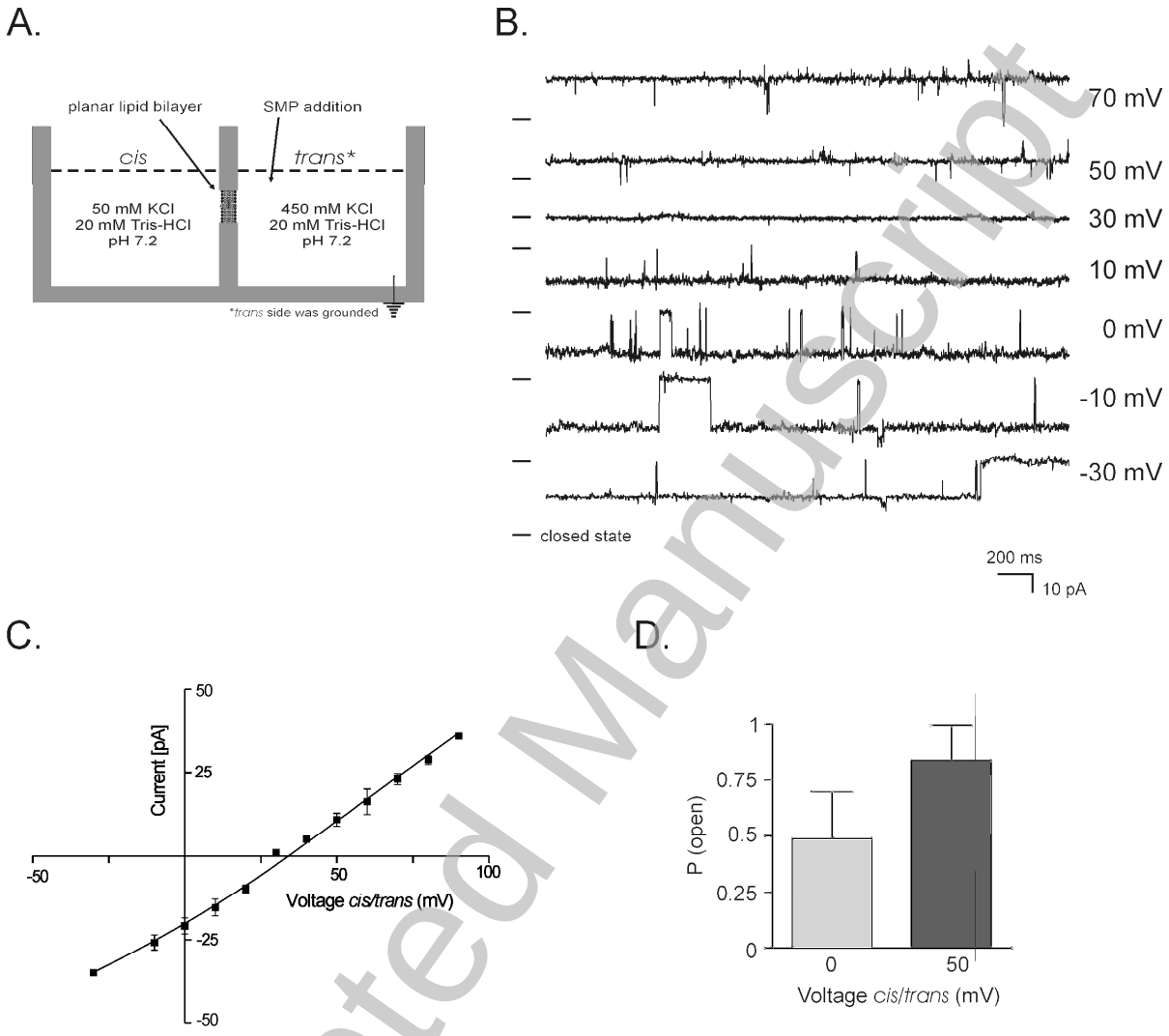


THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/BJ20090991

Accepted Manuscript

Licenced copy. Copying is not permitted, except with prior permission and as allowed by law.
© 2009 The Authors Journal compilation © 2009 Portland Press Limited

Fig. 5.

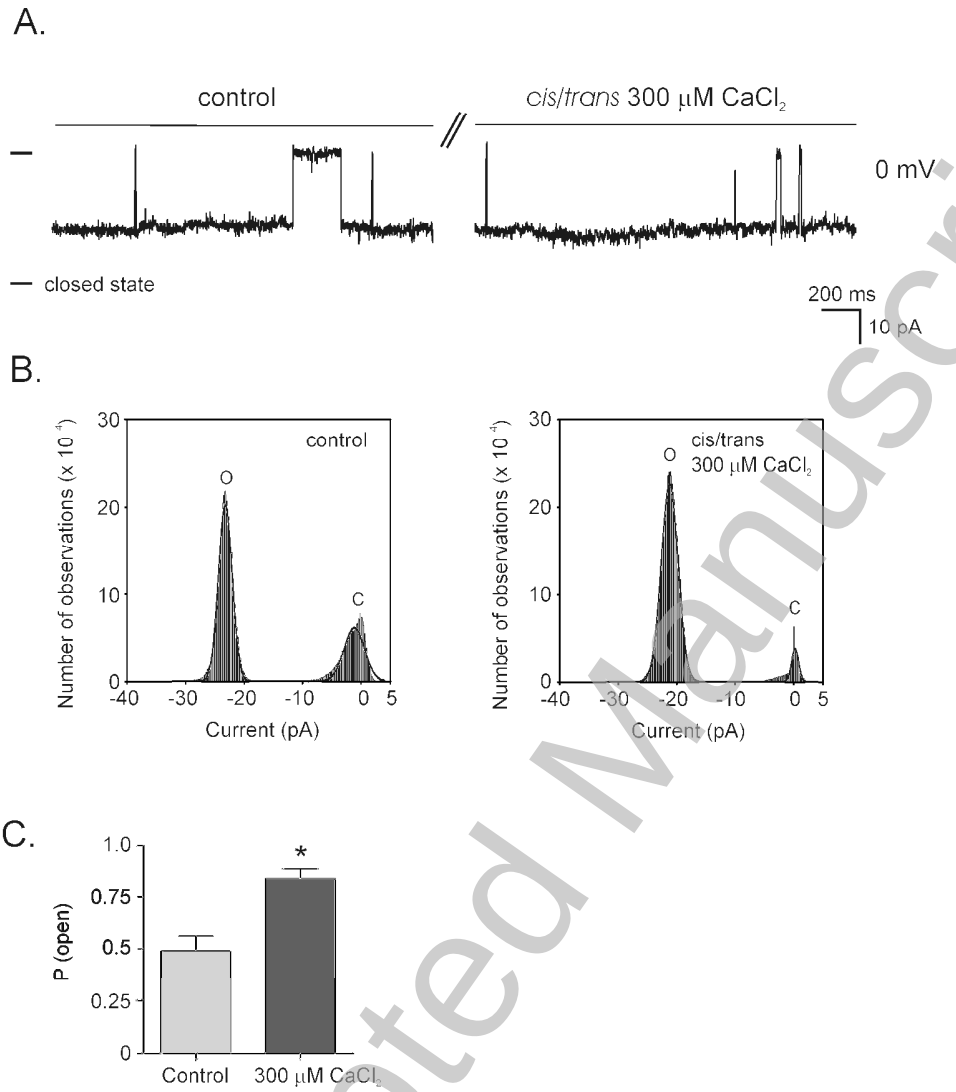


THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/BJ20090991

Accepted Manuscript

Licenced copy. Copying is not permitted, except with prior permission and as allowed by law.
© 2009 The Authors Journal compilation © 2009 Portland Press Limited

Fig. 6.

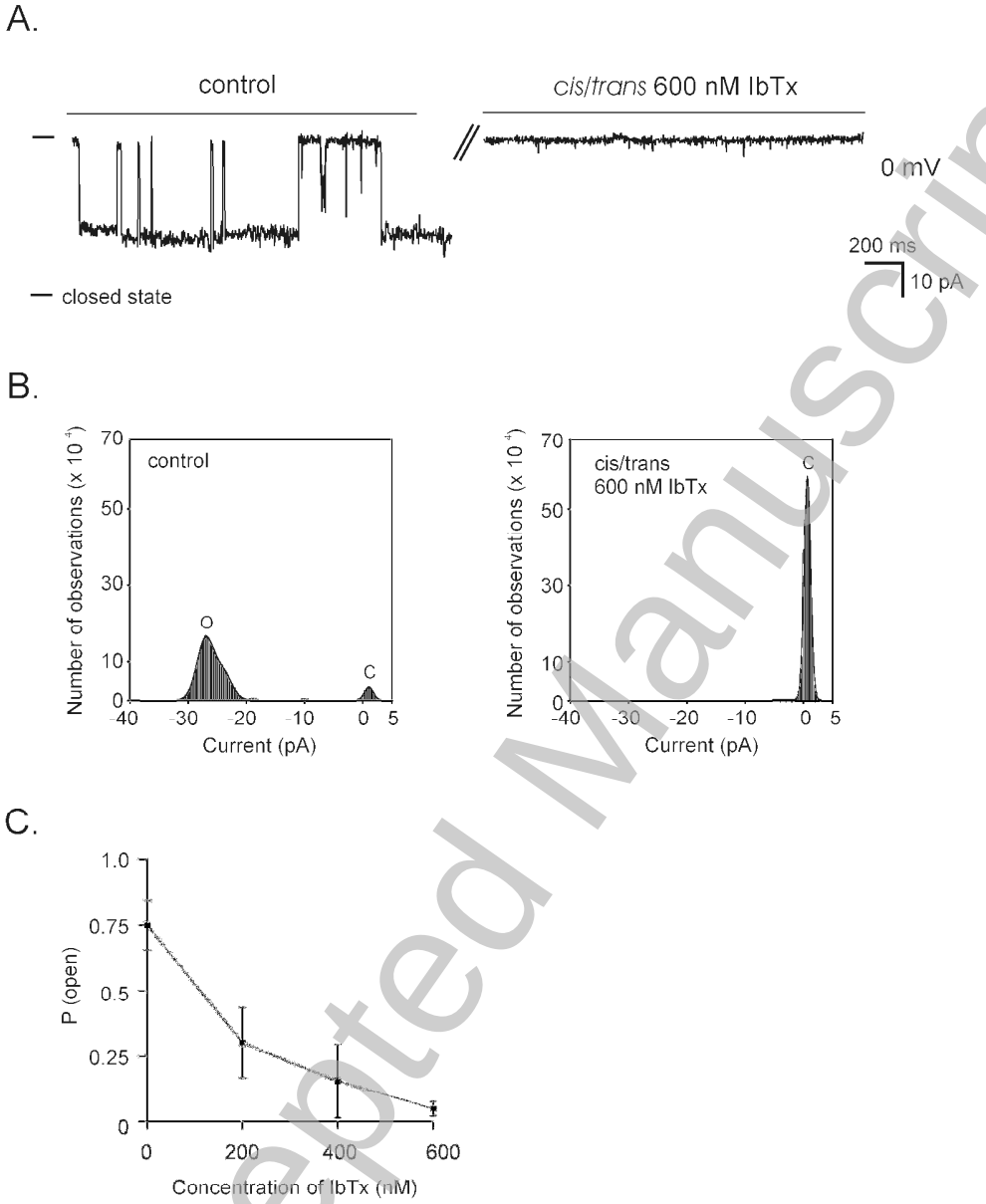


THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/BJ20090991

Accepted Manuscript

Licenced copy. Copying is not permitted, except with prior permission and as allowed by law.
© 2009 The Authors Journal compilation © 2009 Portland Press Limited

Fig. 7

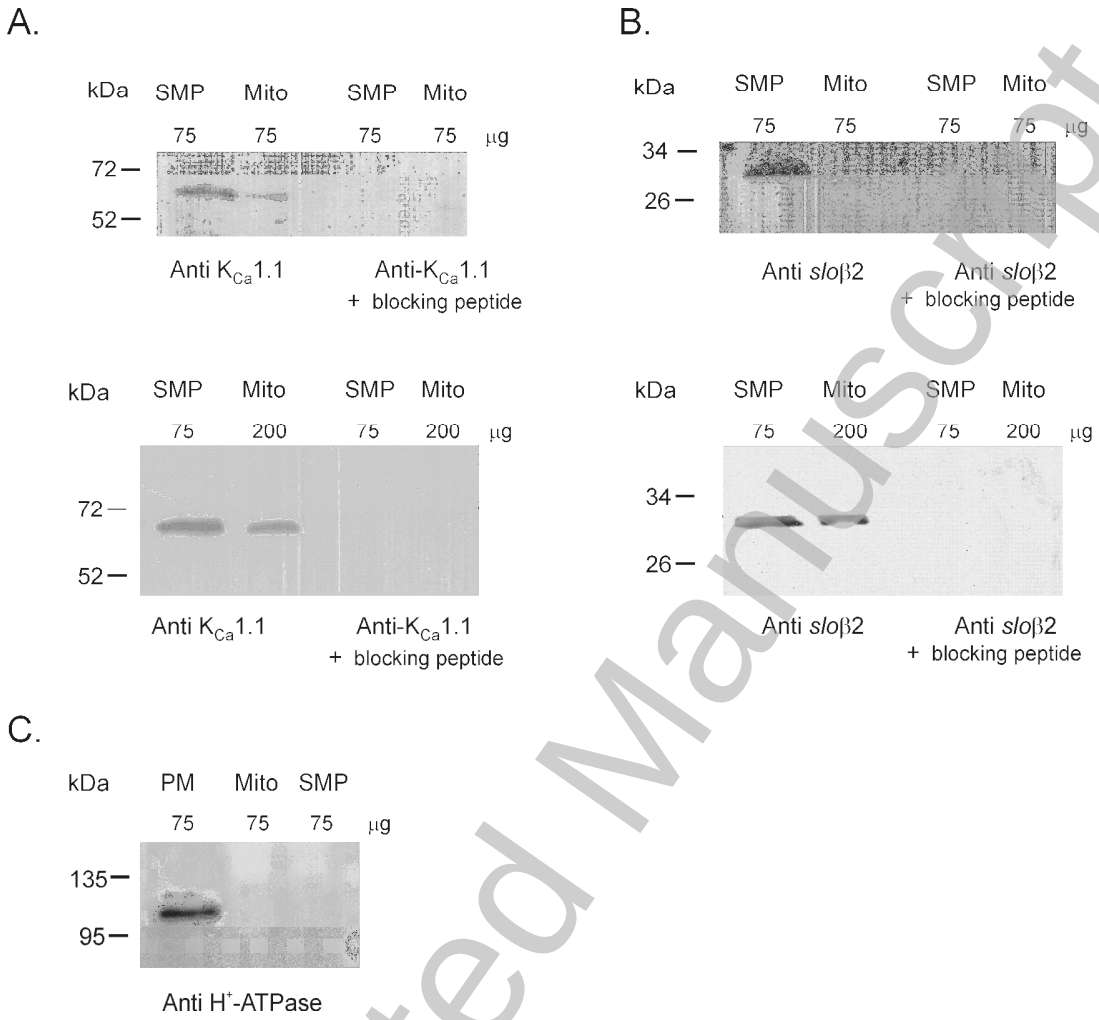


THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/BJ20090991

Accepted Manuscript

Licenced copy. Copying is not permitted, except with prior permission and as allowed by law.
© 2009 The Authors Journal compilation © 2009 Portland Press Limited

Fig. 8.



THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/BJ20090991

Accepted Manuscript

Licenced copy. Copying is not permitted, except with prior permission and as allowed by law.
© 2009 The Authors Journal compilation © 2009 Portland Press Limited