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Electro-pharmacological profile of a mitochondrial inner membrane big-potassium channel from rat brain

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ABSTRACT

Recent studies have indicated a calcium-activated large conductance potassium channel in rat brain mitochondrial inner membrane (mitoBK channel). Accordingly, we have characterized the functional and pharmacological profile of a BK channel from rat brain mitochondria in the present study. Brain mitochondrial inner membrane preparations were subjected to SDS-PAGE analysis and channel protein reconstitution into planar lipid bilayers. Western blotting and antibodies directed against various cellular proteins revealed that mitochondrial inner membrane fractions did not contain specific proteins of the other subcellular compartments except a very small fraction of endoplasmic reticulum. Channel incorporation into planar lipid bilayers revealed a voltage dependent 211 pS potassium channel with a voltage for half activation ($V_{1/2}$) of 11.4 ± 1.1 mV and an effective gating charge z_d of 4.7 ± 0.9. Gating and conducting behaviors of this channel were unaffected by the addition of 2.5 mM ATP, and 500 nM charybdotoxin (ChTx), but the channel appeared sensitive to 100 nM iberiotoxin (IbTx). Adding 10 mM TEA at positive potentials and 10 mM 4-AP at negative or positive voltages inhibited the channel activities. These results demonstrate that the mitoBK channel, present in brain mitochondrial inner membrane, displays different pharmacological properties than those classically described for plasma membrane, especially in regard to its sensitivity to iberiotoxin and charybdotoxin sensitivity.

1. Introduction

Mitochondria are involved in various processes essential for cell survival, including energy production, redox control, calcium homeostasis, and physiological cell death mechanisms. The integrity of mitochondrial membranes is essential to insure the biophysical basis of these complex phenomena [1]. Potassium channels similar to those present in the plasma membrane, including ATP-regulated potassium channels [2], large conductance Ca-regulated potassium channels [3], intermediate conductance Ca-regulated potassium channels [4], voltage-gated potassium channels [5] and twin-pore potassium channels [6] have been found in inner mitochondrial membrane. It has been proposed that mitochondrial potassium channels are involved in the volume regulation in mitochondria [7,8], cytoprotection [9], acidification [7], apoptosis [10], and control of inner mitochondrial membranes' integrity [11].

A putative mitochondrial large conductance Ca^{2+}-activated potassium channel (mitoBK channel) was first described by Siemen et al. [3] in human glioma cells LN229 using patch-clamp technique. The presence of a channel with properties similar to the plasma membrane BK channel was also observed in cardiac mitochondria [10,12] where it could play a prominent role in protecting the heart against ischemic injury [10,13]. Morphological evidence was similarly provided for the presence of a large conductance BK channel in the neuronal inner mitochondrial membrane of rat brain [14]. It was proposed that brain mitoK channels could play an important role in response to hypoxic cell injury. In this regard, Cheng et al. [15] has found that hypoxia increased the mitoBK activity of mitoplasts from rat liver and astrocytes and suggested this response could be interpreted as an anti-apoptotic activity. Notably, activation of mitoBK being localized in the inner membrane of brain mitochondria was reported to inhibit ROS production by respiratory chain complex I [16]. This effect is likely to explain the beneficial effects of BK potassium channel openers on neuronal survival. More recently, it has been observed that Ca-induced mitochondrial membrane potential

Abbreviation: EGTA, ethylene glycol-bis (2 amino-ethylether)-*N,N,N',N'*-tetraacetic acid; HEPES, 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid, N-(2-Hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid) potassium salt; BSA, bovine serum albumin; mitoBK, mitochondrial big Ca^{2+} -activated potassium channel; mitoK$_{ATP}$, mitochondrial ATP-sensitive K^+ channel; Trizma base, Tris [hydroxymethyl] aminomethane; cox, cytochorom oxydase antibody; IbTx, iberiotoxin; ChTx, charybdotoxin; TEA, tetra-ethyl ammonium; 4-AP, 4-aminopyridine; SDS-PAGE, sodium dodecyl sulfate-polyacryl-amide gel electrophoresis; 58KGP, 58K Golgi protein

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depolarization and respiration can be blocked by iberiotoxin and charybdotoxin [17], while providing single channel evidence for a charybdotoxin-sensitive potassium channel. Depending on the cell type, different BK channels can be observed. This diversity is a consequence of alternative splicing and interaction with regulatory subunits which modulate biophysical and pharmacological properties of BK channels. For example, Meera et al. [18] showed that the human BK channel β-subunit (β_4), highly expressed in brain, renders the BK channel α-subunit resistant to ChTx and IbTx [18]. Notably, the presence of a BK channel β4 subunit has been documented in brain mitochondria [14,19]. Finally, TEA resistant BK channels have been identified in guinea-pig myenteric neurons [20] whereas ATP sensitive BK channels were found in renal proximal tubule cells [21].

Potassium channels of the mitochondrial inner membrane are modulated by inhibitors and activators previously described for plasma membrane potassium channels [22] but little is still known about the pharmacology and the molecular identity of brain mitoBK channel. In this study, we show that rat brain mitochondrial inner membrane contains a voltage-gated 211 pS K channel that is insensitive to ChTx and ATP while it is sensitive to IbTx and 4-AP. Besides, it is sensitive to TEA at positive voltages but not at negative voltages.

2. Materials and methods

2.1. Materials

HEPES, sodium bicarbonate, D-manitol, sucrose, digitonin, potassium chloride, Tris–HCl, BSA, nagarase, potassium chloride, EGTA, IbTx, ChTx, ATP, TEA and 4-AP were purchased from Sigma and n-Decane was obtained from Merck. Salts and all solvents were analytical grade.

2.2. Solutions

Solutions for mitochondrial isolation are as follows: MSE-solution (225 mM manitol, 75 mM sucrose, 1 mM EGTA, 5 mM HEPES, 1 mg/ml BSA, pH 7.4); MSE-nagarse solution (0.05% nagarse in MSE solution); MSE-digitonin solution (0.02% digitonin in MSE solution).

2.3. Mitochondria isolation

Mitochondria were isolated from the brain of male Wistar rat (weighing 180–200 g) according to the protocol described by Rosenthal et al. [23] with a small modification. All experiments were done in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1996). In brief, two rats were anesthetized by ether and brains were rapidly removed and homogenized in 20 ml ice-cold MSE-nagarse solution at 600 units/s using potter homogenizer. After adding 40 ml of ice-cold MSE solution, the homogenate was centrifuged at 2000*g* for 4 min. Thereafter, the supernatant (step 1 for western blotting) was centrifuged at 12000*g* for 9 min at 4 ° C (Beckman model J-21B). After dissolving the pellet in 20 ml of ice-cold MSE (step 2 for western blotting) and digitonin, solution was transferred to a 30 ml glass homogenizer, and it was manually homogenized 8–10 times to obtain a homogenous suspension. Subsequently, the suspension was centrifuged at 12000*g* for 11 min, and the obtained pellet was dissolved in 300 µl of MSE solution (35 mg protein/ml) (step 3 for western blotting).

Mitochondrial inner membranes derived from mitochondria were prepared as previously described method [24]. Briefly, mitochondria were suspended in H_2O at a concentration of 5 mg/ml and were stirred for 20 min on ice. The mixture was homogenized 20 times with a glass homogenizer. Then, the suspension was centrifuged twice at 12000*g* for 5 min. Thereafter, the obtained pellet (mitoplasts) were treated with Na_2CO_3 0.1 M, pH 11.5, at a final concentration of 0.5 mg/ml for 20 min on ice. Finally, the suspension was centrifuged at 100000*g* for 30 min. Mitochondrial inner membrane vesicles were stored in 20 µl aliquots in MSE solution, pH 7.4 at −80 ° C until being used (step 4 for western blotting).

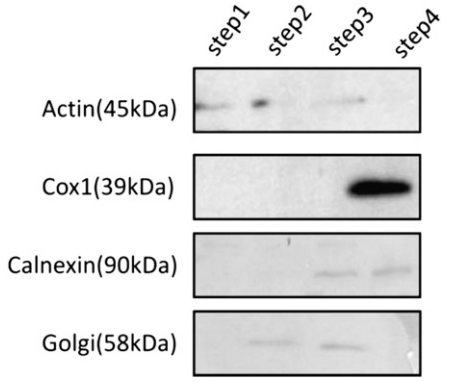

Fig. 1. Purity of cell fractions: western blotting of rat brain. Membranes were probed with organelle specific antibodies. Plasma membrane marker: Actin (C-11); 45 kDa; mitochondrial membrane marker: Cox1 (1D6), 39 kDa; Golgi marker: 58 K Golgi protein; endoplasmic reticulum marker: Calnexin, 90 kDa. Steps are demonstrated in Materials and methods section.

2.4. Immunoblot analysis

2.4.1. Protein samples

Protein concentrations of subcellular fractions were assayed using a DC Protein Assay Kit (Bio-Rad). Protein samples (30 µg) of each

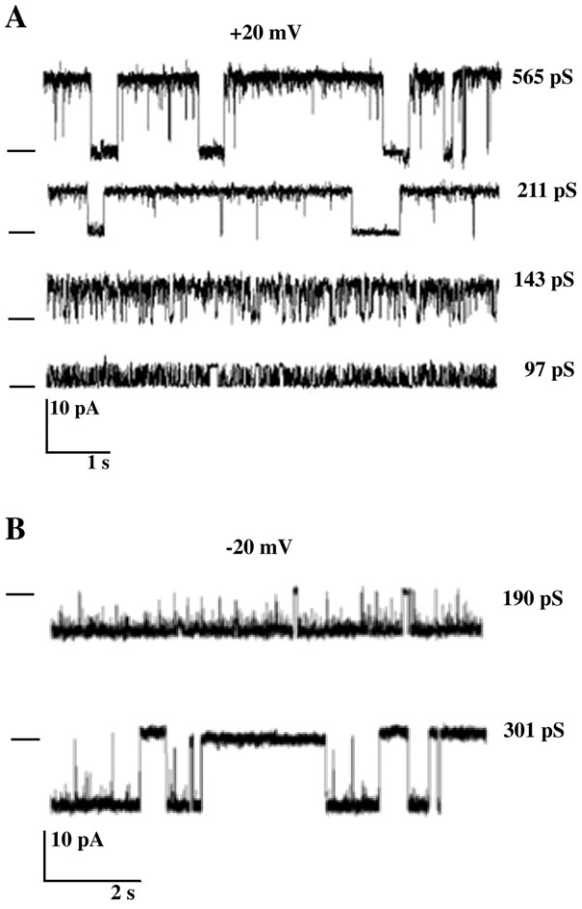

Fig. 2. Single channel recordings of ion channels of brain mitochondrial inner membranes in planar lipid bilayer. (A) Single channel recordings of four different potassium channels at +20 mV and (B) two chloride channels at −20 mV in a 200/50 mM KCl (*cis*/*trans*) gradient solution.