Research Paper

Voltage Activation of Heart Inner Mitochondrial Membrane Channels

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The patch clamp records obtained from mitoplast membranes prepared in the presence of a calcium chelator generally lack channel activity. However, multiconductance channel (MCC) activity can be induced by membrane potentials above ±60 mV [Kinnally et al., Biochem. Biophys. Res. Commun. 176, 1183–1188 (1991)]. Once activated, the MCC activity persists at all voltages. The present report characterizes the activation by voltage of multiconductance channels of rat heart inner mitochondrial membranes using patch-clamping. In some membrane patches, the size of single current transitions progressively increases with time upon application of voltage. The inhibitor cyclosporin has also been found to decrease channel conductance in steps. The results suggest that voltage-induced effects which are inhibited by cyclosporin A are likely to involve either an increase in effective pore diameter or the assembly of low-conductance units. In activated patches, we have found at high membrane potentials (e.g., 130 mV) changes in conductance as high as 5 nS occurring in large steps (up to 2.7 nS). These were generally preceded by a smaller transition. Similar results were obtained less frequently at lower voltages. These results can be explained on the assumption that once assembled the channels may act in unison.

KEY WORDS: Inner mitochondrial membrane; channels; voltage activation; assembly; cyclosporin; patch-clamp; permeability transition pore.

INTRODUCTION

Evidence for high-conductance channel activity with multiple substates (MCC) in mouse liver mitochondria has been obtained using patch-clamp techniques (Kinnally et al., 1989; Petronilli et al., 1989, for review see Kinnally et al., 1992). It has been suggested that MCC activity is responsible for the Ca2+-activated permeability transition observed in mitochondrial suspensions (Kinnally et al., 1991; Szabó and Zoratti, 1991, 1992). In our hands, MCC activity was observed if the mitoplast preparation was done in the presence of (endogenous) Ca2+ (Kinnally et al., 1991). Alternatively, if mitoplasts were prepared in the presence of a calcium chelator and MCC was electrically silent, it could be activated by applying a membrane potential of sufficient magnitude. Once activated, the MCC activity was recorded at all voltages with transitions ranging from 40 to as much as 2,600 pS (Zorov et al., 1991) in rat heart inner mitochondrial membranes. One of the possible explanations for the activation process is that the higher conductance levels result from the assembly of lower conductance channels. Previously, we have reported with activated MCC (Zorov et al., 1991): (a) negative voltage steps induced occupancy of progressively higher conductance levels, whereas positive voltage steps induced occupancy of progressively lower conductance levels, and (b) the introduction of the inhibitor amiodarone induced occupancy of corresponding lower conductance levels. The present study confirms

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some of the characteristics of high-conductance transitions of activated MCC and examines the initial events of voltage activation of MCC in membrane patches initially lacking channel activity (Kinnally et al., 1991).

MATERIALS AND METHODS

Preparation of the Mitoplasts

Hearts from male rats of the Sprague–Dawley strain (100–250 g) were homogenized using a glass and Teflon homogenizer of the Potter–Elvehjem type in a medium containing 230 mM mannitol, 70 mM sucrose, 5 mM HEPES, and 1 mM EGTA, pH 7.4. Large mitochondria were isolated as previously described (Bowman and Tedeschi, 1983), and mitoplasts were prepared from these mitochondria using the French-press method of Decker and Greenawalt (1977). After sedimentation of large mitochondria, the pellet was resuspended in 15 ml of 460 mM mannitol, 140 mM sucrose, and 10 mM HEPES, pH 7.4, kept on ice for 10–15 min and then subjected to 2,000 psi using the French press to remove the outer membrane. The mitoplasts were diluted by an equal volume of 230 mM mannitol, 140 mM sucrose, 5 mM HEPES, and 1 mM EGTA, pH 7.4, kept on ice for 5–10 min. They were centrifuged at 10,000 g for 5 min and resuspended in 3 ml of 150 mM KC1 and 5 mM HEPES, pH 7.4. The procedures used (see Kinnally et al., 1989) produced preparations yielding current records with no significant channel activity when clamped at or below ±60 mV. We refer to these as “silent membrane patches.”

Patch Clamping

About 10 μl of mitoplast suspension was placed on a glass slide. After several minutes the mitoplasts attached to the slide were washed with the patching medium containing 150 mM KC1, 5 mM HEPES, 1 mM EGTA, and 0.95 mM CaCl2 (about 6 × 10−7 M free Ca2+), pH 7.4, at room temperature (approximately 25°C). In this study only excised mitoplast membrane patches were studied. These were presumed to be in the inside-out configuration, since channel activity had the same voltage dependence in attached or excised patches (see Kinnally et al., 1989). The pipettes contained the same medium and their resistance was 20 to 40 MΩ. The reference electrode consisted of a Ag–AgCl wire connected to the bath through a bridge containing the medium in 2% agar. Patch pipettes were formed from 1.0 mm diameter capillary Pyrex tubes (Corning 7740 glass, World Precision Instruments, Inc. New Haven, Connecticut 06513) using a horizontal puller (Sutter Instruments Co. Model PC-84).

A Dagan 3900A patch clamp amplifier in the inside-out mode was used under voltage clamp conditions. The current (bandwidth of 10 kHz) and voltage outputs were digitized with an Instrutech VR-10 digital data recorder (Mineola, New York) and recorded on video tape. Subsequent computer analysis of stored current signals was done at a bandwidth of 2–4 kHz obtained with a low-pass filtering device (Frequency Devices, Haverhill, Massachusetts 01830, model 902) with a sampling frequency at least twice that of the cut-off filter. The computer analysis of the data used Strathclyde Electrophysiological Data Analysis software (courtesy of J. Dempster, University of Strathclyde, UK) with a 2801A D/A board from Data Translation (Marlboro, Massachusetts). Typically, cyclosporin was delivered in a solution of the usual medium, by perfusion of 3 ml through a 0.5 ml bath. Controls were carried out by the same procedure but without the drug and no effect was observed. The quantity nP0 was calculated from amplitude histograms from the ratio of percent time at open current levels over the total time. In this paper, upward deflections are openings with positive voltages and downward deflections are openings with negative voltages.

RESULTS

MCC (multiple conductance channel) activity is characterized by the observation of multiple conductance levels and a nS peak conductance. The presence of MCC activity requires either isolation in the absence of a calcium chelator or activation generally at voltages higher than ±60 mV. The activity may involve more than one channel, and the transitions range from 40 to 2,600 pS. While we observe transitions above 500 pS in over 50% of heart mitoplast patches (n = 25 randomly selected), transitions above 1 nS are seen in about 20% of the membrane patches. More infrequently, we have seen single transitions as high as 5 nS. Besides the inactive, electrically silent form of MCC, we have observed three distinct patterns of activity: (a) conventional opening and closing at various levels; we have recognized a minimum of nine