Journal of Bioenergetics and Biomembranes, Vol. 19, No. 4, 1987

MINI-REVIEW

Channels in the Mitochondrial Outer Membrane: Evidence from Patch Clamp Studies

Henry Tedeschi[1,2] and Kathleen Walsh Kinnally[1]

Received February 13, 1987

Abstract

Patch clamp techniques were applied to outer mitochondrial membranes of giant mitochondria from mice kept on a cuprizone diet or to vesicles produced by fusing membranes derived from the outer membrane of *Neurospora* mitochondria. In the negative range of potentials the conductances decreased with increases in the magnitude of voltage, suggesting the closing of channels. Experiments in which mitochondria were treated with the polyanion polymethacrylate maleate styrene (1:2:3) or succinic anhydride suggest that the channels correspond to VDAC. Although sometimes conductance also decreased with increasing potential over a narrow range of positive potentials, more commonly the conductances increased. Although this phenomenon may represent a detachment of the patch, the changes in conductance are reversible, suggesting that they correspond to the formation or the opening of channels.

Key Words: Mitochondrial outer membrane; mitochondrial channels; channels.

The history of the study of channels has generally followed a particular pattern. First the physiological and pharmacological behavior of the native membranes was throughly studied. The presence of channels of a defined ion specificity, voltage, and drug sensitivity provided the most direct explanation of the results of these studies. The task of isolating the proteins of the channels from membranes followed, and eventually the incorporation of the proteins into bilayers and the characterization of the reconstituted systems. The history of the outer mitochondrial membrane voltage-dependent,

[1]Department of Biological Sciences and Neurobiology Research Center, State University of New York at Albany, Albany, New York 12222.
[2]To whom correspondence should be addressed.

0145-479X/87/0800-0321$05.00/0

anion-selective channel, VDAC, is quite different. As discussed in other papers of this conference, the channels have been recognized and studied extensively with little knowledge of the behavior of the native membrane. These studies are now so advanced that we can already hazard informed guesses on the structure and gating mechanism of VDAC. This is a remarkable tribute to the sagacity of the scientists in this field and to the technology of the bilayer. Hopefully we can now begin to provide the missing information on the behavior of the native membrane by applying the technology of the patch clamp to the study of the outer mitochondrial membrane (Tedeschi *et al.*, 1987).

The use of patch clamp techniques (Neher and Sakman, 1976; Hamill *et al.*, 1981) allows the electrical or actual isolation of a portion of a membrane by fusing a fire-polished pipette to the surface. The technique can be used only with vesicles which have a much larger surface area than the aperture of the pipette, which generally has a tip opening diameter of approximately 1.5–2 μm. The giant mitochondria used in previous studies (Bowman and Tedeschi, 1983) or fused vesicles derived from the outer mitochondrial membrane of *Neurospora crassa* (Mannella, 1982) have the required size.

The application of patch clamping to the study of the outer mitochondrial membrane has distinct consequences which differ significantly from those of studies using bilayers. Since the technique is used with the native membrane, the system under study is much more complex and so it might not be unexpected for the results to differ from those obtained with purified VDAC and bilayers. Perhaps one of the most significant differences is the concentration of channels, which is much greater in the patches. At high VDAC concentrations only a few hundred channels may insert per square millimeter in a bilayer, whereas in an outer membrane patch there are probably hundreds and perhaps a few thousands per square micrometer.

The patches are very leaky because of the presence of many channels, so that the resistance of a patch will be relatively low, under our conditions approximately 100 to 500 MΩ. In addition, the experiments are easier to carry out at low ionic strength, to keep in the range of current most convenient for the use of the patch clamp equipment, so that we have generally used a buffered sucrose medium containing 10 mM KCl. For this reason the pipette resistance, which is in-series with the membrane, is a significant component of the total. Therefore, in order to determine the voltage clamped across the membrane, it was necessary to introduce a correction which was carried out electronically in most of the experiments discussed here.

The geometry of the patch in relation to the pipette could be of considerable significance. Generally, a piece of the membrane is picked up by the pipette in such a way that part of the membrane parallels the side of the patch

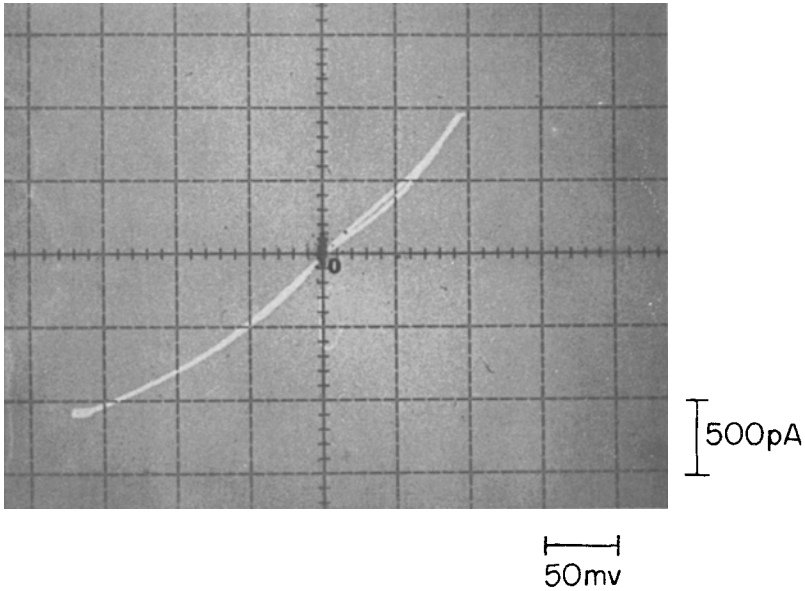

Fig. 1. Oscilloscope tracing of current (ordinate) as a function of voltage (abscissa). The origin (0 current, 0 voltage) is in the center of the figure. The record was obtained by manually increasing the magnitude of the clamped voltage and then returning to 0. The curve showing lower currents in the positive range correspond to the values obtained when returning to 0.

electrode. Accordingly, for this portion, the electric field will be parallel to the plane of the membrane. Since charged proteins can electrophoretically migrate in the plane of a membrane in response to the electric field (Sowers and Hackenbrock, 1981), it becomes necessary to test whether any particular effect of an imposed voltage may be attributed to the migration of channels in the plane of the membrane into or perhaps out of the patch. The evidence which will be discussed later does not support this alternative as long as VDAC are responsible for the observed membrane conductance.

Results obtained with this technique using a giant mitochondrion are shown in Fig. 1. This is a photographic record of an oscilloscope tracing of current (ordinate) as a function of voltage (abscissa) across an outer membrane patch. The sign of the potential in all our records corresponds to the polarity of the inside of the pipette. The origin, representing zero current and voltage, is in the center of the record. The same data are replotted in Fig. 2 in terms of conductance vs. potential. There is clearly a decrease in the conductance with the magnitude of the voltage in the negative range of potential. In the positive range of potential the relationship between current and voltage is biphasic. The conductance first decreases with potential, but

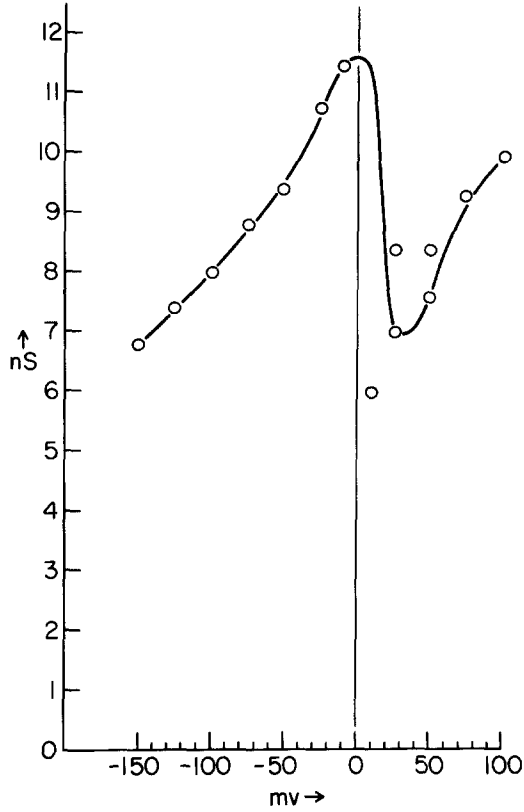

Fig. 2. The results of Fig. 1 plotted as conductances as a function of voltage. The presence of two current values at the same voltage reflect the hysteresis shown in Fig. 1.

then it increases. A decrease in conductance with increasing magnitude of potential either in the positive or the negative range of voltage is characteristic of VDAC as studied in bilayers. That these results indicate the behavior of VDAC in the membrane patch is supported by similar observations obtained with fused vesicles prepared from outer mitochondrial membranes of *Neurospora* (Tedeschi *et al.*, 1987).

The pattern of electrical response shown in Figs. 1 and 2 occurs in patches of giant mitochondria about 5–10% of the time. Generally only an increase in conductance is detected with increasing positive potential. The events in this range of potential may be interpreted as the balance between two independent processes, one closing VDAC with increasing potential, as in the negative range, and the other, an independent process at the moment of unknown significance which causes increased membrane conductance.

Such a large increase in conductance could be the result of the patch becoming detached from the pipette. In experiments without the in-series correction the conductance of the patch plus the electrode occassionally approximates the conductance of the electrode alone. There are, however, several arguments against this interpretation, the most potent being the reversibility of the effect. For example, when positive voltage pulses are delivered in rapid succession, the membrane conductance recovers fully between pulses and the time course of the conductance with each pulse is approximately equal (Tedeschi *et al.*, 1987).

We have examined in some detail whether the decrease in patch conductance observed with negative potentials is consistent with the closing of VDAC, or whether some other mechanism might be involved. One alternative possibility was the electrophoretic migration of channels into or out of the patch in regions normal to the electric field vector. The isoelectric point of VDAC polypeptide is known. Depending on the system, it ranges from a p*I* of 7.7 (Freitag *et al.*, 1982) to 7.9 (Lindén *et al.*, 1982). Therefore the voltage dependence of electrophoresis-based conductance changes at pH values above and below the p*I* should be very different since the net charge of the VDAC protein would be opposite in sign. Bowen *et al.* (1985) have already shown that the electric characteristics of VDAC are relatively insensitive to pH in the range used in these experiments. A comparison of the *IV* curves at pH values above and below the p*I* shows no significant variation (Tedeschi *et al.*, 1987). Therefore, if the voltage-dependent conductance changes involve VDAC, it is highly unlikely that electrophoretic migration plays a role.

Apart from the resemblance between our results and those obtained with VDAC, what is the evidence that VDAC are involved in the voltage-sensitive decrease in conductance? In trying to answer this question we have relied on treatment with compounds previously used in the study of VDAC. The polyanion polymethacrylate maleate styrene (1 : 2 : 3) has been shown to increase the voltage-sensitive closing of VDAC (Yeung *et al.*, 1986). In addition, Doring and Colombini (1985) have shown that treatment of VDAC with succinic anhydride under some conditions virtually eliminates the response of the channels to voltage. A typical result showing the effect of the polyanion is depicted in Fig. 3. These results, together with the more complete study already published (Tedeschi *et al.*, 1987), clearly show that the voltage sensitivity of the mitochondrial patches is greater in the presence of the polyanion. The treatment with succinic anhydride also has the expected result, i.e., the decrease in patch conductance with increasing negative voltage is eliminated (Tedeschi *et al.*, 1987).

From all these experiments we would like to conclude that we have been working with the outer mitochondrial membrane and that the decrease in

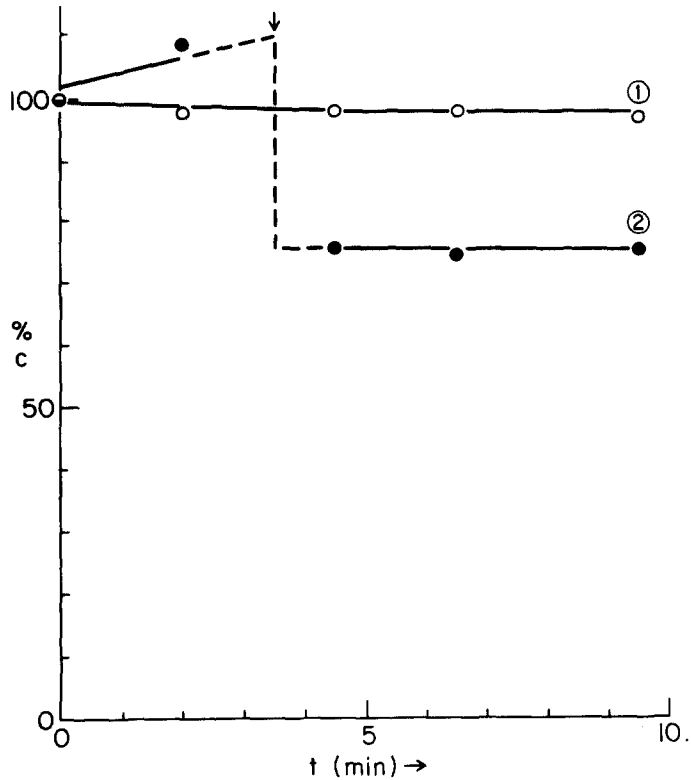

Fig. 3. Effect of addition of the polyanion. Curve 1 represents a typical control; 200 μl of the suspension medium were added at the arrow to 50 μl of suspension. In the experimental (curve 2) 200 μl of suspension medium containing the polyanion to a final concentration of 42 μM were introduced. The results shown for each curve were obtained using the same patch. The voltage was clamped at −75 mV. The results are expressed as percent conductance. Unpublished experiments of Tedeschi *et al.* (1987).

conductance with voltage in both the negative and positive potential range corresponds to the closing of the VDAC.

Another feature of the behavior of the patches in relation to the effect of the polyanion merits further discussion. Normally, we found two populations of patches observed in mitochondria before the addition of the polyanion, responsive and relatively unresponsive to voltage. However, after the addition of the polyanion, a single responsive population was evident (Tedeschi *et al.*, 1987). Yeung *et al.* (1986) have proposed that there might be a physiological equivalent to the polyanion which confers voltage sensitivity on VDAC. Our observations support this view. The patches which respond to voltage in the absence of added polyanion might contain the endogenous factors while unresponsive mitochondria might lack them. The addition of

the polyanion simply replaces the endogenous factor when it has been lost so that all mitochondrial patches respond.

The increase in conductance in the positive range of potentials appears to be a novel phenomenon. At this time we cannot eliminate the possibility that the patch is becoming detached, although the reversibility of the effect strongly argues against this conclusion. The increase in conductance is also sensitive to the polyanion although this compound increases rather than decreases the conductance.

Acknowledgments

The research summarized in this discussion was carried out in collaboration with Charles L. Bowman and Carmen Mannella and was supported by ONR grant N0001485K0681 to one of us (HT) and NSF grant PCM-8315666 to Carmen Mannella.

References

Bowen, K. A., Tam, K., and Colombini, M. (1985) *J. Membr. Biol.* **86**, 51–59.
Bowman, C. L., and Tedeschi, H. (1983). *Biochim. Biophys. Acta* **731**, 261–266.
Doring, C., and Colombini, M. (1985). *J. Membr. Biol.* **83**, 81–86.
Freitag, H., Neupert, W., and Benz, R., (1982). *Eur. J. Biochem.* **123**, 629–636.
Hamill, O. P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F. J. (1981). *Pfluger Arch.* **391**, 85–100.
Lindén, M., Gellerfors, P., and Nelson, B. D. (1982). *Biochem. J.* **208**, 77–82.
Mannella, C. (1982). *J. Cell. Biol.* **94**, 680–687.
Neher, E., and Sakman, B. (1976). *Nature* (*London*) **260**, 799–802.
Sowers, A. E., and Hackenbrock, C. R. (1981). *Proc. Nat. Acad. Sci. USA* **78**, 6246–6250.
Tedeschi, H., Bowman, C. L., and Mannella, C. (1987). *J. Membr. Biol.* **97**, in press.
Yeung, C. L., Tung, J., Colombini, M., and Konig, T. (1986). *Biophys. J.* **49**, 206A.