Journal of Bioenergetics and Biomembranes, Vol. 29, No. 3, 1997

Effects of Carbonyl Cyanide Phenylhydrazones on Two Mitochondrial Ion Channel Activities

Maria Luisa Campo,[1] Henry Tedeschi,[2] Concepción Muro,[1] and Kathleen W. Kinnally[2,3]

Received September 30, 1996; accepted December 21, 1996

The respiratory uncouplers carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) affect the activities of two mitochondrial ion channels from mouse liver. At micromolar concentrations, the phenylhydrazones block the voltage-dependent 100-pS channel, mCS, and induce the multiple-conductance-level channel, MCC. The binding site(s) involved in perturbation of channel activities are probably distinct from the sites involved in uncoupling of oxidative phosphorylation which occurs at nanomolar concentrations of the phenylhydrazones. The effects of FCCP and CCCP on the mitochondrial ion channels could be partially reversed by washing with fresh media and were always reversed by perfusion with dithiothreitol. These results indicate that the effects of the phenylhydrazones on mitochondrial ion channels may be related to the ability of these compounds to act as sulfhydryl reagents and not to their protonophoric and uncoupling activity.

KEY WORDS: Mitochondria; uncouplers; channels; patch-clamp; MCC; mCS.

INTRODUCTION

Patch-clamp studies of mitoplasts (mitochondria with the inner membrane exposed) reveal the presence of two large-conductance ion channel activities (reviewed in Kinnally *et al.*, 1996; Sorgato and Moran, 1993; Zoratti and Szabó, 1995). The mitochondrial *c*entum pico*s*iemen channel, mCS, is voltage dependent and slightly anion selective, displaying a conductance of ~100 pS at physiological salt concentrations (Sorgato *et al.*, 1987). The *m*ultiple *c*onductance *c*hannel activity, MCC, is voltage dependent and slightly cation selective with a peak conductance of 1–1.5 nS (Kinnally *et al.*, 1992, 1996; Lohret and Kinnally, 1995a). This megachannel recently has been linked to protein import by the effect of targeting peptides on its open probability (Lohret and Kinnally, 1995b). Both MCC and mCS are normally quiescent in mitoplast patches but can be differentially activated during mitochondrial isolation (Kinnally *et al.*, 1991, 1992).

Different classes of metabolic effectors of mitochondria are being screened in an attempt to characterize the functional roles of these ion channel activities. In a previous study, it was determined that the electron-transport inhibitor antimycin A inhibited mCS and MCC activities at low micromolar concentrations (Campo *et al.*, 1992). The present communication reports the effects of the oxidative-phosphorylation uncouplers FCCP and CCCP[4] on the channel activities of mouse-liver mitoplasts recorded using patch-clamp techniques. In this study, we surveyed for direct effects of the phenylhydrazones on these voltage-dependent channel activities under voltage-clamp conditions rather than for indirect effects associated with depolarization of the membrane potential. A

[1] Departamento de Bioquímica y Biologia Molecular y Genética, Universidad de Extremadura, Cáceres, Spain.
[2] Department of Biological Sciences, University at Albany, SUNY, Albany, New York.
[3] Department of Biomedical Sciences, Division of Molecular Medicine, Wadsworth Center, Empire State Plaza, P.O. Box 509, Albany, New York 12201-0509.
[4] Abbreviations: Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone, FCCP; Carbonyl cyanide *m*-chlorophenylhydrazone, CCCP; Dithiothreitol, DTT; mitochondrial Centum picoSiemen channel, mCS; Multiple Conductance Channel activity, MCC.

0145-479X/97/0600-0223$12.50/0

preliminary report of these findings has been made (Campo *et al.*, 1995).

MATERIALS AND METHODS

Mitochondria with mCS and/or MCC activity were isolated from 21- to 35-day-old mice by a modification of the method of Campo *et al.* (1992). One liver was manually homogenized with four strokes with a Teflon pestle, brought to a final volume of 11 ml with 230 mM mannitol, 70 mM sucrose, 1 mM EGTA, 5 mM HEPES, pH 7.4 (medium I), and centrifuged at 110 × *g* for 80 s. Each 5 ml of supernatant was layered on 5 ml of 460 mM mannitol, 140 mM sucrose, 1 mM EGTA, 10 mM HEPES, pH 7.4 (medium II), and centrifuged at 800 × *g* for 3 min. The top layer and interface were then centrifuged at 2,000 × *g* for 5 min. The mitochondrial pellet was resuspended in 5 ml of medium II containing 0.75 mM $CaCl_2$ (≈0.1 μM free Ca^{2+}) and incubated on ice for 10 min. Mitochondria were subjected to French pressing at 2,000 psi according to Decker and Greenawalt (1977). The resulting mitoplasts were diluted with three volumes (15 ml) of medium I containing 0.75 mM $CaCl_2$ (≈0.1 μM free Ca^{2+}) and centrifuged at 2,600 × *g* for 10 min. The pellet was resuspended in 2 ml of 150 mM KCl, 1 mM EGTA, 2 mM $MgCl_2$ 0.75 mM $CaCl_2$ (≈0.1 μM free Ca^{2+}), 5 mM HEPES, pH 7.4, and 2.5 μM rotenone (Medium III). $MgCl_2$ and rotenone were omitted from medium III in some experiments with no apparent effect. Mitoplasts with little or no ion channel activity ("electrically silent" preparations) were prepared using isolation media in which calcium was omitted. In general, mitoplasts with channel activity were prepared with calcium present in the isolation media and were used in inhibitor studies while electrically silent preparations were used for inducer studies.

Electrophysiological measurements were made on patches excised from mitoplasts at room temperature in medium III. Current data were examined at voltages of ±40, ±30, ±20, and ±10 in the presence and absence of phenylhydrazones. Additions were made by perfusion with a minimum of 5 ml through a 1-ml chamber. Stock solutions of FCCP (Sigma C2920) and CCCP (Sigma C2759) in ethyl alcohol were prepared monthly and stored at −20°C as aliquots. Liposomes were prepared from purified soybean L-α-phosphatidylcholine (Sigma Type IVs) as previously described (Lohret and Kinnally, 1995a), using the method of Criado and Keller (1987) except that ethylene glycol was omitted.

Voltage clamp was performed with the inside-out excised configuration of the patch-clamp technique (Hamill *et al.*, 1981) using a Dagan 3900 patch clamp amplifier in the inside-out mode. Voltages across patches excised from mitoplasts are reported as bath (i.e., matrix) potentials. Microelectrodes (W.P.I., New Haven Connecticut, 1b100f-4) were pulled to a tip diameter of ~0.3 μM (Sutter Instrument model PC84, Novato, California) and had a resistance of 10–30 mΩ (program courtesy of A. K. Dean). Current data were recorded at 10 kHz and low-pass filtered during subsequent analysis at 2 kHz (Frequency Devices, Haverhill, Massachusetts, model 902) unless otherwise specified. Data were analyzed with the PAT program (courtesy of J. Dempster, U. Strathclyde, UK). Open probabilities, P_0, were computed from total amplitude histograms as the fraction of time spent in the open-current level. In patches with more than one channel, the time spent occupying level *k* was fit to a binomial equation for open probability calculations [%Time = $[P^k(1 - P)^{(n-k)}]$ $[n!/k!(n - k)!]$ courtesy of H. Frisch, Chem.

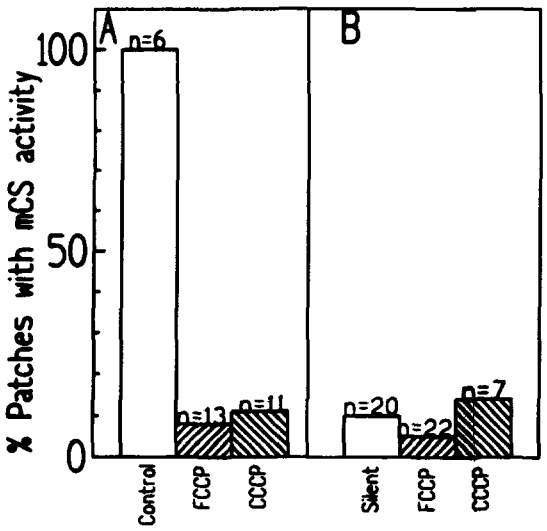

Fig. 1. FCCP and CCCP inhibit mCS activity. (A) The percent of the independent patches (*n*) that maintained at least half the initial open probability of mCS was determined at low positive potentials after perfusion with media containing 0.2–100 μM FCCP or CCCP or an equivalent amount of ethanol (control). (B) The percent of patches with mCS activity was determined after incubation of "electrically silent" mitoplasts suspensions with 40 μM FCCP or CCCP or an equivalent amount of ethanol (silent) for 45 min on ice. Furthermore, perfusion of excised patches initially exhibiting no mCS activity with media containing FCCP (8 patches), CCCP (7 patches), or an equivalent amount of ethanol (6 patches) did not induce mCS activity (not shown).

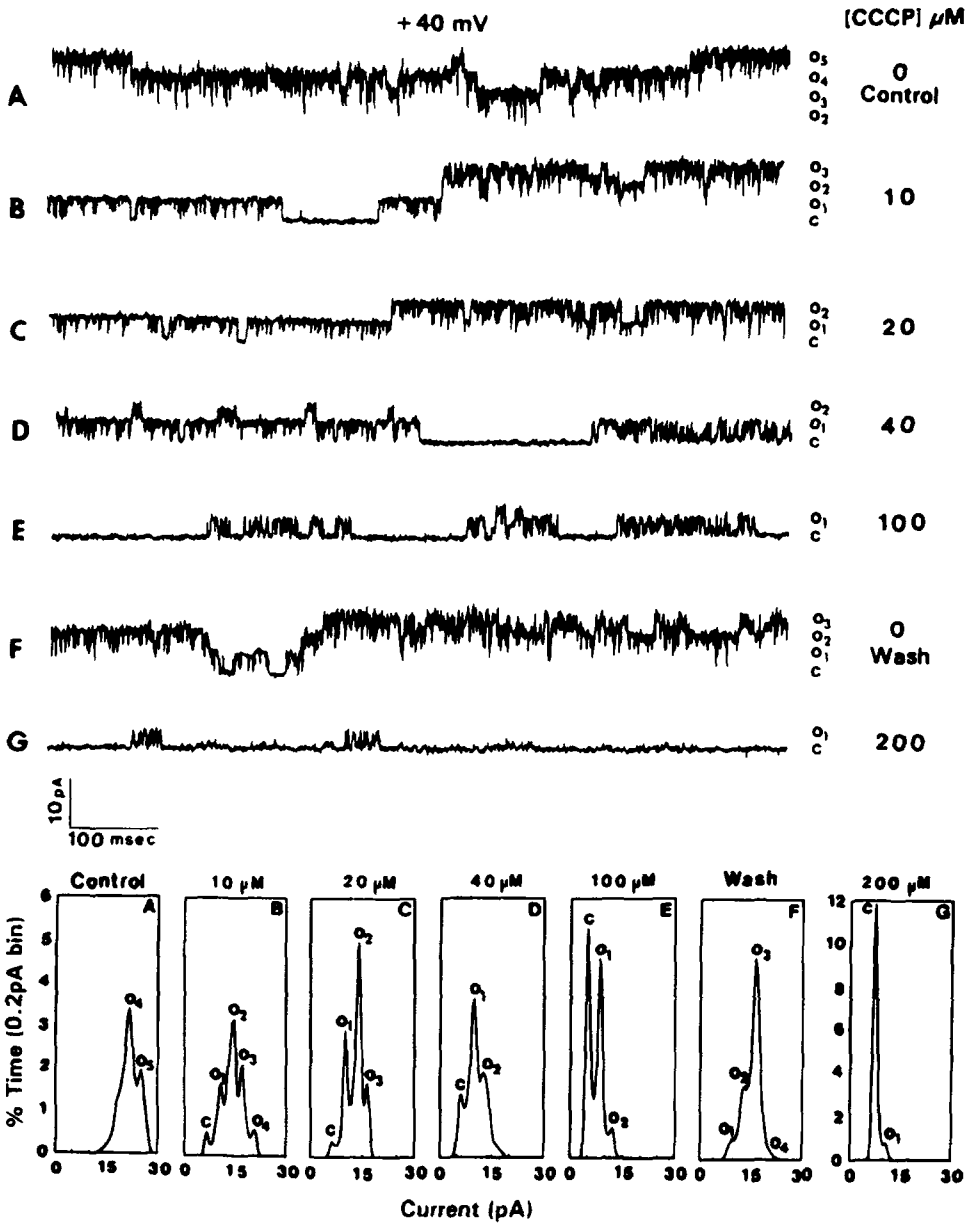

Fig. 2. CCCP inhibits mCS activity. Typical current traces (top) and total current amplitude diagrams (bottom) are shown for a mitoplast patch voltage-clamped at 40 mV and treated sequentially with (A) 0 μM CCCP, (B) 10 μM CCCP, (C) 20 μM CCCP, (D) 40 μM CCCP, (E) 100 μM, (F) 0 μM CCCP, and (G) 200 μM CCCP. C, O_1, O_2, O_3, O_4, and O_5 correspond to the current levels of the closed state and one, two, three, four, and five open channels, respectively. Current amplitude diagrams were computed over 40–60 s of data. The P_0 was 0.7 prior to the addition of CCCP.

SUNY Albany, Albany, New York], where P is probability and n is number of channels. Current levels in amplitude histograms are not corrected for leaks. Single-channel analysis was done at 2 kHz with 5-kHz sampling. Gating charge and V_0, voltage, at which P_0 is 0.5, were calculated as previously reported (Lohret and Kinnally, 1995a) according to Moczydlowski (1986). Burst lengths were determined from current traces (~60 s duration) using a 5-ms maximum closed interval for termination. Mean closed times were the average duration of the closed times. Values of half-maximal effective concentrations (mean ± standard

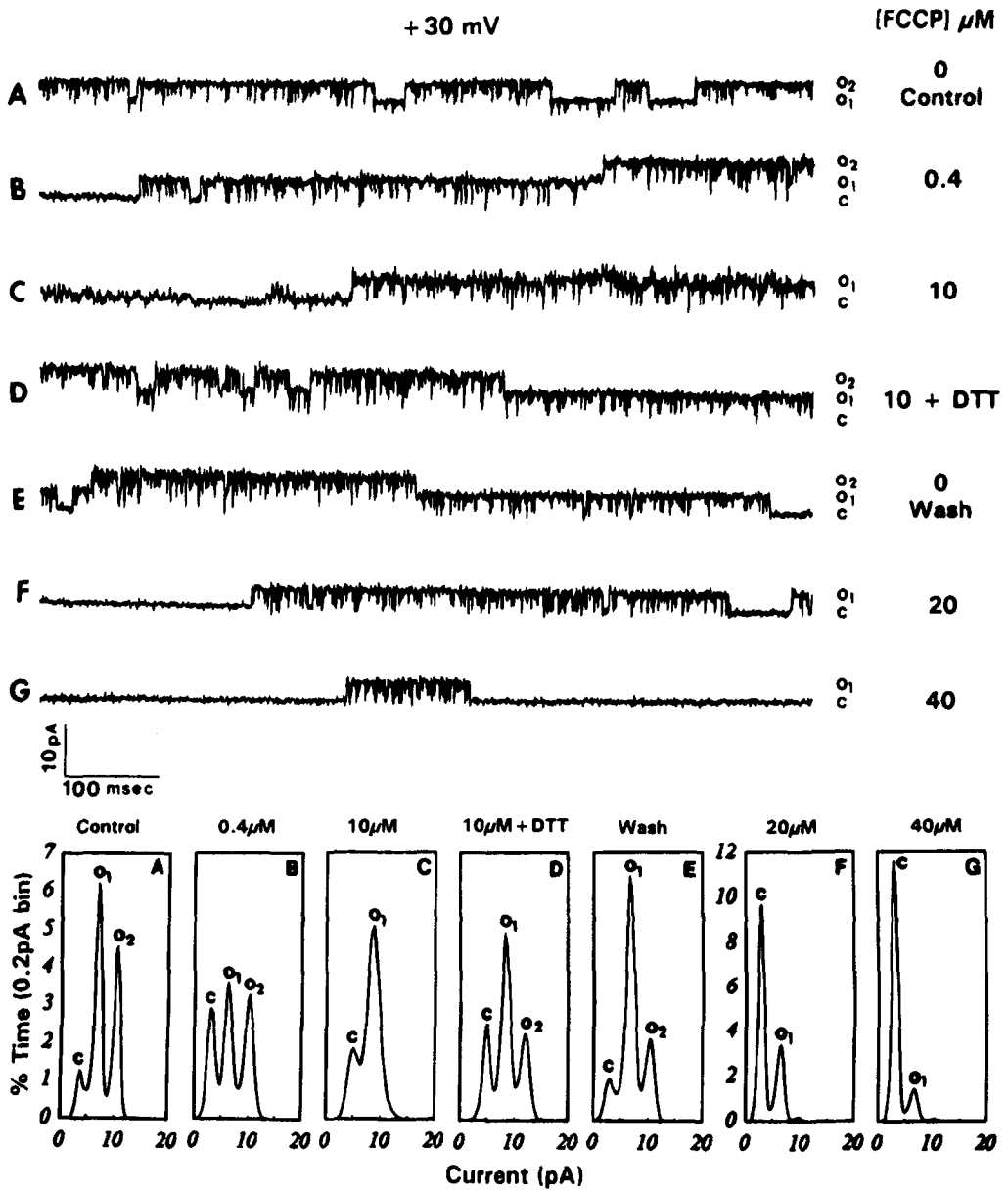

Fig. 3. FCCP inhibits mCS activity. Typical current traces (top) and total current amplitude diagrams (bottom) are shown for a mitoplast patch at 30 mV that was treated sequentially with (A) 0 μM FCCP, (B) 0.4 μM FCCP, (C) 10μM FCCP, (D) 10μM FCCP plus 1 mM DTT, (E) 0 μM FCCP, (F) 20 μM FCCP, and (G) 40 μM FCCP. The P_o was 0.5 prior to the addition of FCCP. Other conditions are as in Fig. 2.

error) were determined for a minimum of four independent patches.

RESULTS

Carbonyl Cyanide Phenylhydrazones Inhibit mCS Activity

The mitochondrial channel mCS is voltage dependent and slightly anion selective with a conductance of ~100 pS (Sorgato *et al.*, 1987). Like other inner-membrane channels with large conductance, mCS is quiescent unless activated, in this case, by varying free calcium and divalent cation chelator levels (Kinnally *et al.*, 1991). The effect of phenylhydrazones on mCS activity of 100 independent patches from murine liver mitoplasts is summarized in Fig. 1.

Micromolar levels of phenylhydrazones inhibited mCS activity in ~90% of the excised patches initially

displaying this activity (Figs. 1A, 2, and 3). Also, phenylhydrazones did not induce mCS activity since the frequency of detecting mCS in "electrically silent" preparations of mitoplasts is unchanged by pretreatment with 40 μM phenylhydrazone or by perfusion with up to 200 μM phenylhydrazone (Fig. 1B). The progressive inhibition of five mCS channels with increasing CCCP levels from 10 to 200 μM is shown in the current traces and corresponding current amplitude diagrams of Fig. 2. Similarly, the open probability of mCS was blocked slightly by 10μM FCCP and almost completely by 40 μM FCCP as shown in Fig. 3. FCCP and CCCP displayed about the same dose-dependent inhibition of normalized mean current levels (Fig. 4). The concentration needed to decrease the open probability to half the control level is 30 ± 12 μM for FCCP (mean ± S.E., 13 patches) and 26 ± 7 μM for CCCP (5 patches).

mCS normally displays bursting behavior, i.e., many openings are grouped together separated by brief closed periods, as shown in Fig. 2 and 3. At low positive potentials, bursts of openings are typically terminated by a closure of 5 ms or longer. Further channel analysis revealed that micromolar levels of phenylhydrazone significantly reduced the duration of bursts in a concentration-dependent fashion (Fig. 5A). Similarly, micromolar levels of phenylhydrazone significantly increased the mean closed times of mCS at low positive potentials as illustrated by the effect of FCCP in Fig. 5B. Phenylhydrazones shift the V_0 to greater positive potentials for mCS activity with no apparent effect on gating charge (data not shown).

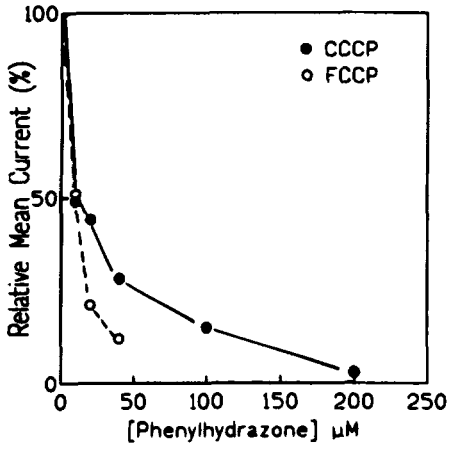

Fig. 4. Inhibition of mCS activity by FCCP and CCCP is dose-dependent. The mean current level (40–70 s duration) at varied concentrations of FCCP (○) at 30 mV and of CCCP (●) at 40 mV was normalized to the control mean current level after leak subtraction and shown as a percent of the relative control level.

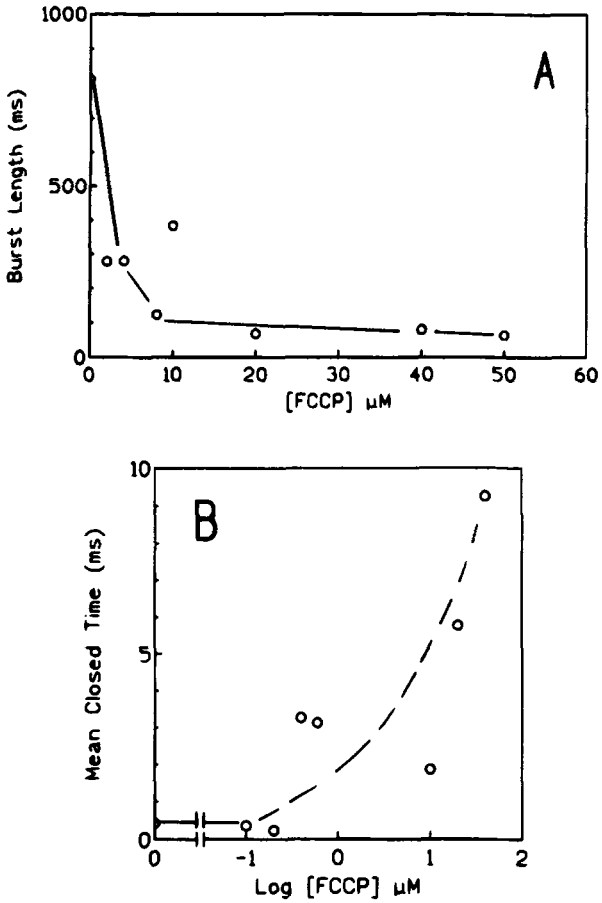

Fig. 5. FCCP modifies mCS burst length and mean closed time. (A) Burst lengths and (B) mean closed times for mCS were determined from current traces at 30 mV at varied FCCP concentrations. Other conditions as in Figs. 2 and 4.

The inhibition of mCS by phenylhydrazones was partially reversed in four of nine patches by perfusion with media lacking phenylhydrazone (Fig. 2F) and completely reversed in six of six patches perfused with phenylhydrazone plus 1 mM DTT (Fig. 3D).

In control experiments, repetitive perfusion of mitoplast patches with media lacking phenylhydrazone (but in some cases, containing an equivalent amount of ethanol) had no effect on mCS activity (6 of 6 patches). Furthermore, phenylhydrazones in concentrations of 0.1–100 μM had no effect on the conductances of patches excised from liposomes devoid of protein in the voltage range of ± 50 mV (3 of 3 patches).

Carbonyl Cyanide Phenylhydrazones Activate MCC Activity

MCC is a voltage-dependent channel of the mitochondrial inner membrane with a peak conductance of

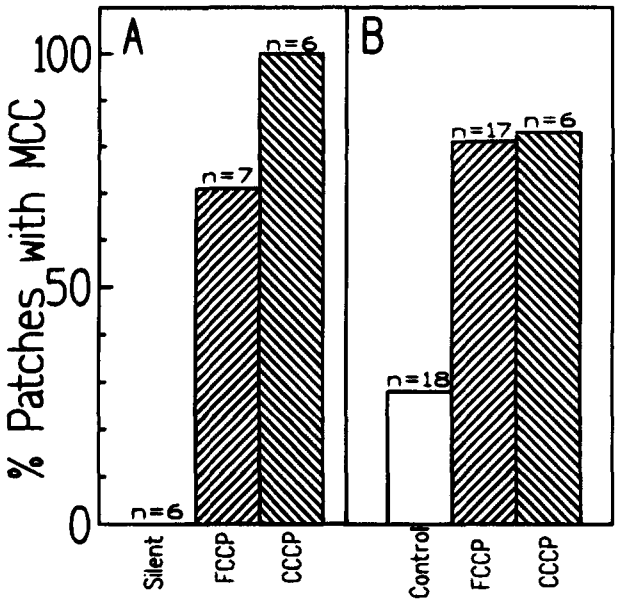

Fig. 6. FCCP and CCCP induce MCC activity. (A) The percent of "electrically silent" patches displaying MCC activity was determined after perfusion of the matrix face with media without (silent) or with phenylhydrazone at 30 mV. Patches scored as negative for MCC activity displayed linear current–voltage plots and lacked MCC transitions (300–1500 pS). (B) The percent of patches with MCC activity was determined after mitoplast suspensions that were initially "electrically silent" were incubated for 45 min on ice in the absence (control) or presence of 40 μM FCCP or CCCP.

1–1.5 nS (Kinnally *et al.*, 1992, 1996; Lohret and Kinnally, 1995a). Like mCS, MCC is quiescent unless activated, e.g., by high transmembrane potentials ($|V| > 60$ mV) (Zorov *et al.*, 1992a,b). The effect of phenylhydrazones on the MCC activity of 60 mitoplast patches is summarized in Fig. 6.

Micromolar levels of phenylhydrazones induced MCC activity (Figs. 6–8). Perfusion of previously "electrically silent" patches with phenylhydrazones resulted in the activation of MCC in 11 of 13 patches at low potentials (±40 mV) (Fig. 6A). The induction of MCC was progressive as the concentration was increased from 0 to 5 μM CCCP as shown by the current traces and corresponding current amplitude histograms of Fig. 7. A similar activation of MCC by 20 μM FCCP is shown in Fig. 8. Furthermore, pretreatment of suspensions of mitoplasts with 40 μM phenylhydrazone increased the frequency of detecting MCC from 28% to over 80% of the pretreated patches (Fig. 6B). Once activated by phenylhydrazones, MCC displayed the same voltage dependence as it does when induced by other agents, e.g., calcium. In general, the presence of MCC in patches scored inactive in the voltage range of ±40 mV was verified by activation of MCC by higher voltages ($|V| \geq 60$ mV). About the same concentration of the two phenylhydrazones was needed to induce the half-maximal conductance of MCC; 11 ± 6 μM FCCP (6 patches at 30 mV) and 11 ± 8 μM CCCP (4 patches at 40 mV).

The activation of MCC (like the inhibition of mCS) by phenylhydrazones was partially reversed in three of seven patches by perfusion with media lacking phenylhydrazones (Fig. 7) and completely reversed in six of six patches after perfusion with media containing excess (1 mM) DTT and micromolar phenylhydrazone (e.g., Fig. 8).

DISCUSSION

The phenylhydrazones FCCP and CCCP inhibit the activity of the mCS channel and induce MCC activity in mouse liver mitoplasts. The concentrations for half-maximal effects are similar for both phenylhydrazones, i.e., ~25 μM for mCS inhibition and ~10 μM for MCC activation at low positive potentials. Since the frequency of detecting MCC at low potentials ($|V| \leq 40$ mV) increases in the presence of phenylhydrazones, the induction of MCC may be due either to a shift to lower activating potentials for MCC (Zorov *et al.*, 1992a,b) and/or a destabilization of the quiescent closed state. The behavior (e.g., burst length and mean open time) induced by phenylhydrazones at low positive potentials mimics that recorded in controls at negative potentials which is consistent with a shift in V_0 (see Campo *et al.*, 1992).

The large standard deviation observed in the concentrations of phenylhydrazones needed for half-maximal effects on mCS and MCC may be attributable to the large variations in V_0 reported for mCS (Campo *et al.*, 1992) and/or to partitioning of the hydrophobic phenylhydrazones into membranes (Ramsden, 1993; Dreisbach *et al.*, 1993). This variability also may be related to a lability of the sites of action of FCCP and CCCP.

Uncoupling Action of FCCP and CCCP Is Unrelated to Effects on mCS and MCC

The uncoupling actions of FCCP and CCCP at concentrations of 0.05–0.1 μM are generally thought to be related to their ability to increase the "proton

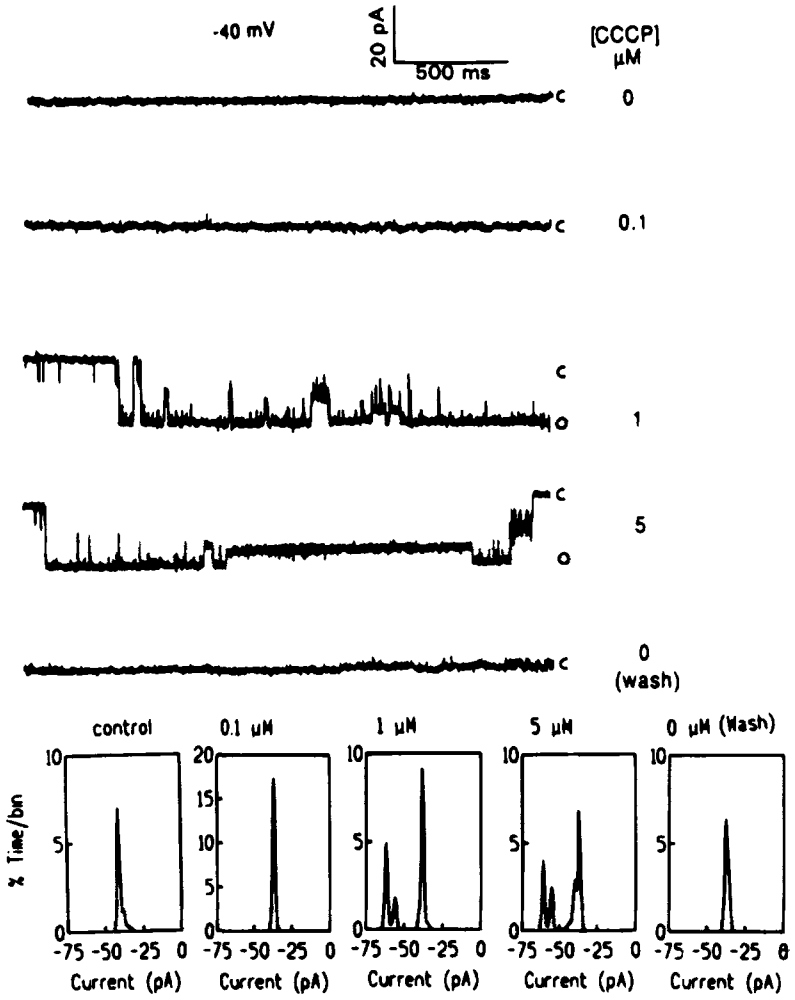

Fig. 7. Reversible induction of MCC by CCCP. Typical current traces (top) and total current amplitude diagrams (bottom) of MCC activity at −40 mV in an excised patch before perfusion (control, O μM CCCP) or after perfusion with media containing the indicated concentration of CCCP. Other conditions as in Fig. 2.

leak" through bilayers (Heytler and Prichard, 1962; Skulachev *et al.*, 1967; Hopfer *et al.*, 1970). Since the reported effects of FCCP and CCCP on mCS and MCC activities occur at much higher concentrations (10–30 μM), they are probably unrelated to the uncoupling effects of phenylhydrazones. Furthermore, pretreatment of intact mitoplasts with phenylhydrazones had the same effects on both mCS and MCC as direct application of phenylhydrazone to excised patches which were artificially depolarized by clamping at low potentials. Therefore, the effects of phenylhydrazones on the mitochondrial channels are probably due to direct interactions with the channel proteins rather than an indirect result of depolarization.

Liberman and Topaly (1968) report that FCCP at 0.02 to 340 μM induced an increase in bilayer conductance of 10^{-8} to 10^{-4} S cm^{-2}. The corresponding maximal change in conductance in the patches (~0.5 μM diameter) used in these experiments would be 0.1–0.2 pS, which is below the limits of resolution in our experiments. Consistent with this, FCCP and CCCP were found to have no effects on the leak conductance of the patches in these experiments.

Possible Involvement of Sulfhydryl Groups

CCCP, FCCP, and other carbonyl cyanide phenylhydrazones are known to bind to sulfhydryl groups

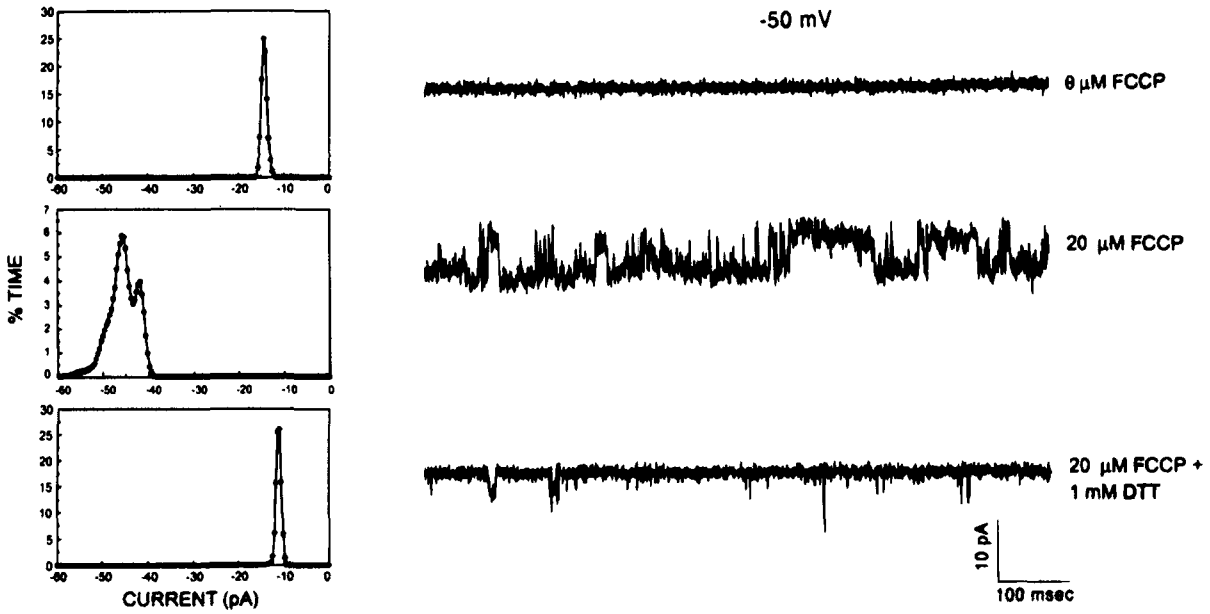

Fig. 8. Reversible induction of MCC by FCCP. Typical current traces (right) and total current amplitude diagrams (left) for an excised patch before perfusion (top) and after perfusion with media containing 20 μM FCCP without (middle) and with (bottom) 1 mM DTT. Other conditions as in Fig. 7.

(Heytler, 1963; Kaback *et al.*, 1974) and to react with aminothiols (Drobnica and Sturdik, 1979; Heber *et al.*, 1979). In general, these reactions can be prevented by the sulfhydryl reductant DTT. Furthermore, phenylhydrazones, such as CCCP and FCCP, are known to react with thiols, like DTT, to form the corresponding N-(substituted phenyl)-N′-(alkylthiodicyano)-methylhydrazine derivatives (Drobnica and Sturdik, 1979).

While (1 mM) DTT had no effect on untreated mCS and MCC activities, it reversed the effects of micromolar levels of phenylhydrazones on both channel activities. This reversal may be due to covalent addition of DTT to the phenylhydrazone thereby rendering them inactive or to reduction by DTT of mitochondrial sulfhydryl groups previously oxidized by the phenylhydrazones. However, the latter interpretation seems inconsistent with the observation that simple washing partially reversed the effects of the phenylhydrazones on the activity of the mitochondrial channels in about half the patches tested. Reversibility of uncoupling effects of phenylhydrazones is somewhat controversial. Initial observations of Heytler (1963) indicated that washing of isolated mitochondria did not reverse uncoupling by phenylhydrazones. However, recent observations by Herrington *et al.* (1996) suggest uncoupling of cells can be reversed by simple perfusion.

Relationship between MCC and the Permeability Transition

DTT was found to inhibit the opening of the permeability transition pore induced in mitochondria by oxidizing agents, e.g., menadione and *tert*-butylhydroperoxide, (Petronilli *et al.*, 1994). The inhibition was attributed to effects of the sulfhydryl reagents on vicinal thiols thought to be associated with the voltage sensor of the transition pore. The possibility that activation of MCC underlies the permeability transition pore has been raised by several groups (Zoratti and Szabó, 1995; Kinnally *et al.*, 1996). Along these lines, it should be noted that uncouplers facilitate opening of the permeability transition pore at nM concentrations (Petronilli *et al.*, 1993) that have little or no effect on MCC.

Effects of FCCP and CCCP on Other Mitochondrial Activities

It has been suggested that the dissipating effects of CCCP on mitochondrial membrane potential are responsible for the observed inhibition of protein import at CCCP concentrations of 10–100 μM (Martin *et al.*, 1991). However, this concentration range is

much higher than that needed to uncouple oxidative phosphorylation and instead is consistent with that needed to affect the activities of the mitochondrial channels. Interestingly, MCC has been implicated as playing a role in protein import based on the channel's transient blockade by targeting peptides (Lohret and Kinnally, 1995b). Similarly, the inhibition of respiration by FCCP has an IC_{50} of 40 μM and is thought to be the result of an inhibition of substrate transport due to energy depletion (Harris, 1968). Additional studies are needed to establish any relationship between mCS and MCC and the inhibition of respiration and protein import induced by phenylhydrazones.

ACKNOWLEDGMENT

This study was supported in part by grants DGICYT PB92-0720 and Junta de Extremadura y Fondo Social Europeo EIA 94/11 and by National Science Foundation grants MCB9117658 and MCB9513439. We thank Dr. Carmen Mannella for support, discussions, and review of this manuscript. We also thank Charles Hauer and Thomas J. Ryan of the Wadsworth Center (Albany, New York) for their helpful discussions.

REFERENCES

Campo, M. L., Kinnally, K. W., and Tedeschi, H. (1992). *J. Biol. Chem.* **267**, 8123–8127.

Campo, M. L., Kinnally, K. W., and Tedeschi, H. (1995). *Biophys. J.* **68**, A144.

Criado, M., and Keller, B. U. (1987). *FEBS Lett.* **224**, 172–176.

Decker, G. L., and Greenawalt, J. W. (1977). *Ultrastruct. Res.* **59**, 44–56.

Dreisbach, A. W., Greif, R. L., Lorenzo, B. J., and Reidenberg, M. M. (1993). *Pharmacology* **47**, 295–299.

Drobnica, L., and Sturdik, E. (1979). *Biochim. Biophys. Acta* **585**, 462–476.

Hamill, O. P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F. J. (1981). *Pflügers Arch.* **381**, 85–100.

Harris, E. J. (1968). *Biochem. J.* **68**, 247–251.

Heber, U., Kirk, M. R., and Boardman, N. K. (1979). *Biochim. Biophys. Acta* **546**, 292–306.

Herrington, J., Park, Y. B., Babcock, D. F., and Hille, B. (1996). *Neuron* **16**, 219–228.

Heytler, P. G. (1963). *Biochemistry* **2**, 357–361.

Heytler, P. G., and Prichard, W. W. (1962). *Biochem. Biophys. Res. Commun.* **7**, 271–275.

Hopfer, U., Lehninger, A. L., and Lennarz, W. J. (1970). *J. Membr. Biol.* **3**, 142–155.

Kaback, H. R., Reeves, J. P., Short, S. A., and Lombardi, F. J. (1974). *Arch. Biochem. Biophys.* **160**, 215–222.

Kinally, K. W., Zorov, D. B., Antonenko, Yu., and Perini, S. (1991). *Biochem. Biophys. Res. Commun.* **176**, 1183–1188.

Kinnally, K. W., Zorov, D. B., and Antonenko, Yu. (1992). *J. Bioenerg. Biomembr.* **24**, 99–110.

Kinnally, K. W., Lohret, T. A., Campo, M. L., and Mannella, C. A. (1996). *J. Bioenerg. Biomembr.* **28**, 115–124.

Liberman, E. A., and Topaly, V. P. (1968). *Biochim. Biophys. Acta* **163**, 125–136.

Lohret, T. A., and Kinnally, K. W. (1995a). *Biophys. J.* **68**, 2299–2309.

Lohret, T. A., and Kinnally, K. W. (1995b). *J. Biol. Chem.* **270**, 15950–15953.

Martin, J., Mahlke, K., and Pfanner, N. (1991). *J. Biol. Chem.* **266**, 18051–18057.

Moczydlowski, E. (1986). In *Ion Channel Reconstitution*, C. Miller, ed. (Plenum Press, New York) pp. 75–113.

Petronilli, V., Cola, C., Massari, S., Colonna, R., and Bernardi, P. (1993). *J. Biol. Chem.* **268**, 21939–21945.

Petronilli, V., Costantini, P., Scorrano, L., Colonna, R., Passamonti, S., and Bernardi, P. (1994). *J. Biol. Chem.* **269** 16638–16642.

Ramsden, J. J. (1993). *Experientia* **49**, 688–692.

Skulachev. V. P., Sharaf, A. A., and Liberman, E. A. (1967). *Nature* (London) **216**, 718–719.

Sorgato, M. C., and Moran, O. (1993). *Crit. Rev. Biochem. Mol. Biol* **28**, 127–171.

Sorgato, M. C., Keller, B. U., and Stühmer, W. (1987). *Nature (London)* **330**, 498–500.

Zoratti, M., and Szabó, I. (1995). *Biochim. Biophys. Acta* **1241**, 139–176.

Zorov, D. B., Kinnally, K. W., and Tedeschi, H. (1992a). *J. Bioenerg. Biomembr.* **24**, 119–124.

Zorov, D. B., Kinnally, K. W., Perini, S., and Tedeschi, H. (1992b). *Biochim. Biophys. Acta* **1105**, 263–270.