Induction of K^+ Transport and Swelling in Isolated Heart Mitochondria by Mercurial Compounds

G. P. Brierley*, C. T. Settlemire, and V. A. Knight

Department of Physiological Chemistry, College of Medicine
Ohio State University, Columbus, Ohio 43210

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Recent studies in our laboratory have established that the mercurial thiol reagent p-chloromercuriphenyl sulfonate (CMS) markedly activates the energy-linked accumulation of Mg^{++} and Pi when ATP is supplied as the energy source (Brierley, et al, 1967). The activation by CMS is enhanced further by the addition of Zn^{++} in the presence of ATP, but the Zn^{++} activated uptake of Mg^{++} which occurs in the presence of respiration is inhibited by CMS (Brierley, 1967). CMS also inhibits oxidative phosphorylation and dinitrophenol-stimulated ATPase activity, but not dinitrophenol-dependent respiration. These and other observatior are compatible with the suggestion that CMS interferes with the transfer of energy from the dinitrophenol-sensitive site to the ion accumulation reaction (Brierley et al, 1967). Inhibition by CMS would infer that a thiol group participates in the energy-transfer sequence at this point. However, inhibition at the suggested point does not account satisfactorily for the increased Mg^{++} uptake observed in the presence of ATP unless additional assumptions are also introduced. We have therefore extended our investigation of the effect of CMS and other SH-group reagents to a number of other energy-linked reactions of the mitochondria. The present communication describes experiments which indicate that CMS and other mercurials can induce a massive uptake of K^+ and a closely related swelling in heart mitochondria. This activation of the energy-linked accumulation

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* Established Investigator of the American Heart Association.

of K^+ occurs in the presence of either respiration or exogenous ATP and appears to establish that under these conditions CMS functions as an activator of mitochondrial ion transport rather than an inhibitor of the energy transport system.

Results - Fig. 1 shows the effect of the addition of CMS to a suspension of heart mitochondria respiring with ascorbate and N, N, N', N' tetramethylphenylenediamine (TMPD) as substrates. After a short lag period the K^+ electrode record shows a marked uptake of K^+ by the mitochondria. There is a simultaneous decrease in absorbance at 546 mμ (swelling). In the absence of the mercurial little uptake of K^+ or swelling occur. The response is both prevented and reversed by uncouplers of oxidative phosphorylation (Fig 1) and inhibitors of

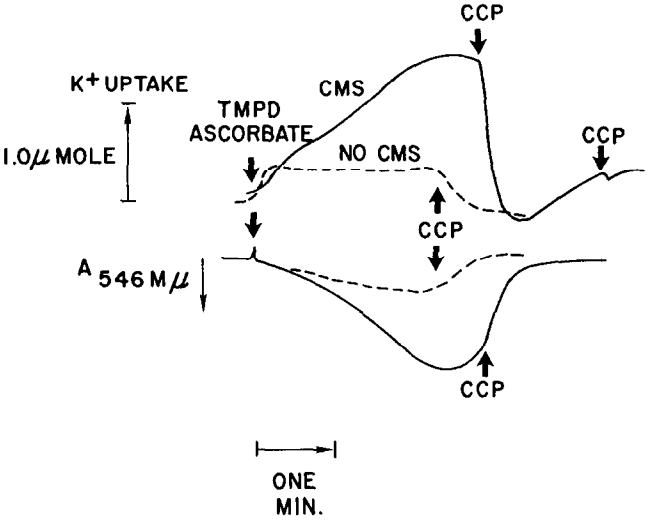

Fig 1 - Induction of K^+ accumulation and swelling of isolated heart mitochondria by p-chloromercuriphenyl sulfonate (CMS). Nagarse beef heart mitochondria (Hatefi, et al, 1961) (10 mg. of protein) were added to 6 ml of a medium consisting of sucrose (150 mM), Tris HEPES (20 uM, pH 7.0), Tris acetate (5 mM, pH 7.0), K^+ acetate (5 mM), Tris phosphate (2 mM), Tris ascorbate (5 mM), TMPD (0.1 mM), and rotenone (7 uM). K^+ uptake was monitored with a Beckman cation sensitive electrode (Pressman, 1965) and swelling was recorded simultaneously with an Eppendorf photometer at 546 mμ using a circular curvette 3/4 inch in diameter and a magnetic stirrer. The solid traces indicate the response to 120 uM CMS; the dashed traces the response in the absence of the mercurial. CCP (20 uM) was added where indicated.

respiration. The requirements of this reaction are summarized in the swelling traces presented in Fig. 2. Initiation of the reaction with CMS results in a lag of about 30 sec. before swelling begins. The reaction requires substrate or ATP and the presence of a permeant anion such as acetate or Pi. Initiation of the reaction with either substrate, acetate, or K^+ shows no lag and indicates that the reaction of the mercurial with the proper site on the mitochondrion is probably responsible for the observed lag period when the reaction is initiated with CMS. CMS initiates little swelling when Na^+ is substituted

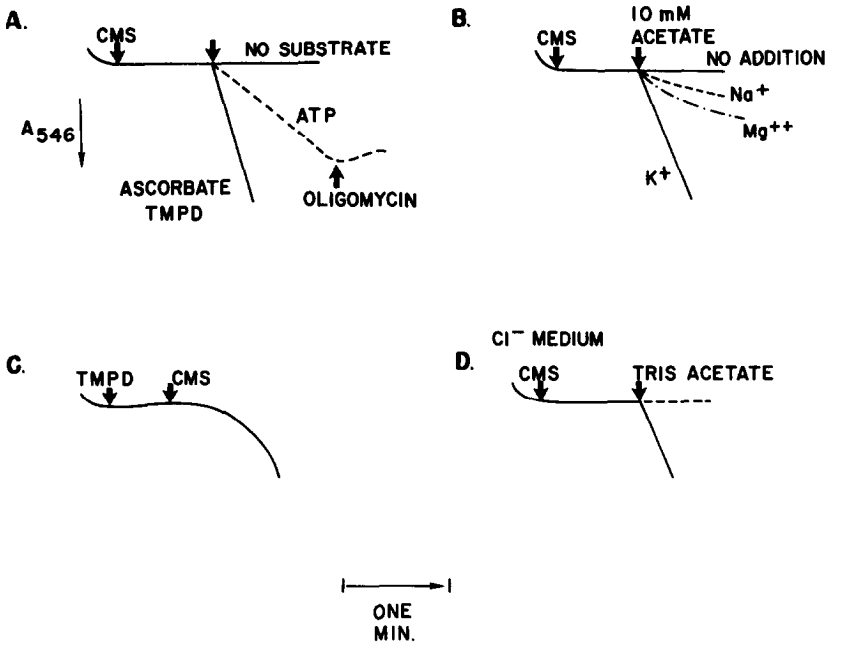

Fig 2 - Properties of CMS-dependent swelling in heart mitochondria. Swelling was monitored with the Eppendorf photometer as described in the legend for Fig 1. Five mg of protein were added to 6 ml of a medium consisting of sucrose (150 uM), K^+ acetate (10 uM), Tris ascorbate (5 mM), TMPD (0.1 mM), and rotenone (7 uM). At the indicated point CMS was added to a concentration of 67 uM. In part A substrate was omitted and added at the indicated point. The dashed trace shows the effect of 3 mM ATP in the absence of respiration and of the addition of oligomycin (0.5 ug/mg) in the presence of ATP. In part B the K^+ acetate was omitted and added at the arrow. The response to K^+ is compared to that seen with no further addition and with the addition of 10 mM Na^+ acetate and with 10 mM Mg^{++} acetate. In Part C the reaction mixture was complete and swelling was initiated by the addition of CMS (67 uM). In part D, K^+ chloride was substituted for the acetate salt and 3 mM Tris acetate added where indicated.

for K^+ (Fig 2). Mg^{++} produces a somewhat greater response than Na^+ under these conditions. Mersalyl and p-hydroxymercuribenzoate substitute for CMS in the role of inducer, but N-ethylmaleimide is much less effective.

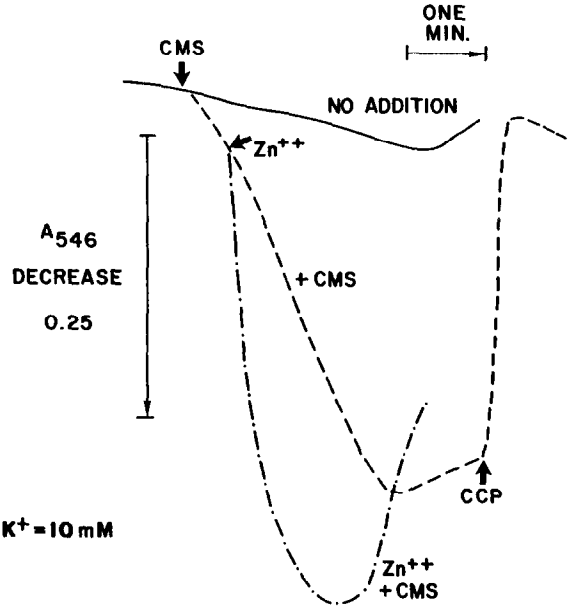

Fig 3 - CMS and Zn^{++}-dependent swelling in a medium containing 10 uM K^+ acetate and 2 mM Tris phosphate. The conditions and suspending medium otherwise were identical to that described in the legend for Fig 2. The concentrations of the additions indicated were CMS (67 uM), Zn^{++} (67 uM), and CCP (20 uM).

The study shown in Fig. 3 establishes that the CMS-dependent swelling supported by TMPD-ascorbate oxidation in a medium containing K^+ and Pi is enhanced by the addition of Zn^{++}. Zn^{++} induces swelling and K^+ uptake under these conditions in the absence of the mercurial (Brierley, et al, 1966; Brierley and Settlemire, 1967). CMS added after the swelling has been initiated by Zn^{++} also enhances the rate of swelling. Both reagents, therefore, appear to activate K^+ transport and K^+-dependent swelling under these conditions and combinations of Zn^{++} and CMS enhance the rate of the reaction.

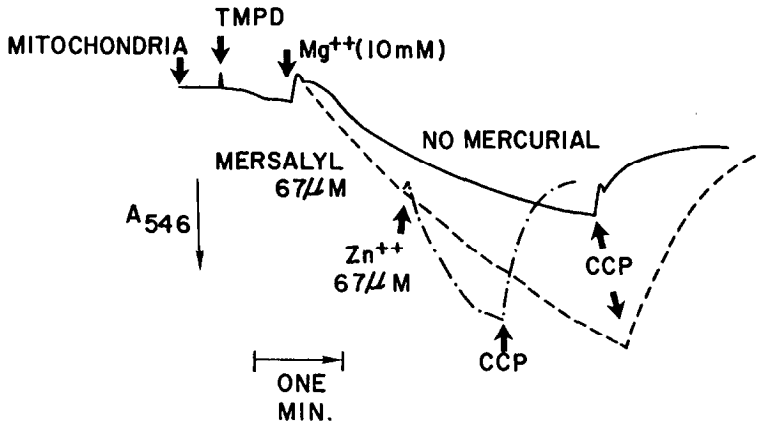

Fig 4 - Mersalyl and Zn^{++}-dependent swelling in a Mg^{++} acetate medium. The conditions of the experiment were identical to those described for the complete system in Fig 2 with the exception that 10 mM Mg^{++} acetate replaced the K^{+} salt. The solid trace shows the response to Mg^{++} addition in the absence of mercurial while the dashed trace shows the effect of 67 uM mersalyl added with the mitochondria. Where indicated Zn^{++} (67 uM) and CCP (20 uM) were also added during the course of the experiment.

In the absence of Pi mercurials also induce a respiration-dependent swelling in the presence of Mg^{++} and acetate (Fig 4). This reaction is enhanced by the addition of Zn^{++}, although rapid aggregation of the mitochondria prevents observation of the swelling after about one minute.

Discussion - These studies establish that CMS and other mercurial reagents markedly activate the energy-linked accumulation of K^{+} and a closely associated K^{+}-dependent swelling in isolated heart mitochondria. The results appear to complement the earlier work of Scott and Gamble (1961) who noted that mercurials profoundly affect the exchange and retention of bound K^{+} by mitochondria. Since the energy-linked ion accumulation reactions reported here can be supported either by exogenous ATP or by ascorbate-TMPD respiration, it appears unlikely that CMS causes a lesion in the mitochondrial energy-transfer system as we have previously suggested (Brierley, et al, 1967). It seems much more likely that the inhibition of Mg^{++} and Pi accumulation supported by ascorbate-TMPD respiration seen in the presence of CMS (cf. Brierley, 1967, Fig 2) is the result of secondary considerations and not inhibition of

energy transfer. Studies presented here (Fig 3 and 4) establish that the combination of Zn^{++} and CMS actually enhances K^{+}-dependent swelling in the presence of Pi, and Mg^{++}-dependent swelling in the absence of Pi when energy is supplied by respiration.

The mechanism of the enhanced ion uptake observed in the presence of mercurials and heavy metals remains obscure and is the subject of continuing investigation. A more complete account of these studies will be presented for publication elsewhere.

Acknowledgements

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