RESPIRATORY CONTROL ASSOCIATED WITH CYCLIC pH TRANSITIONS INDUCED BY N,N'-BIS (DICHLOROACETYL) 1,12-DIAMINODODECANE*

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SUMMARY - Bis-dichloroacetamides induce changes in oxygen uptake which resemble respiratory control. These changes are accompanied by respiratory dependent cyclic pH changes. Strong uncouplers such as CCP which induce similar alkalizations of the medium block the cyclic response to the dichloroacetamides. Swelling in various salt media suggest that the uncoupling-like effect of the drugs is due to an induction of anion permeability and in contrast to the proton conducting uncouplers which are presumed to cause the penetration of protons into the mitochondrion, the dichloroacetamides cause an extrusion of OH^- in weakly buffered media.

For some time we have been investigating the effects of antispermatogenic N,N'- bis-dichloroacetamides on microsomal and mitochondrial function and have found them to be potent inhibitors of drug metabolism (1) as well as inhibitors of mitochondrial pyridine nucleotide-linked electron transport, which also partially uncouple oxidative phosphorylation and stimulate Mg^{++} ATPase activity (2, 3). Furthermore, the effects on the energy linked functions were found to be specific for the dichloro derivative. The acetyl, mono and trichloro derivatives are without activity in these systems (3). In this paper we describe experiments demonstrating a transitory release of State 4 respiration which is accompanied by slight, but reproducible, respiration dependent cyclic pH transitions and suggest that the most probable cause for these uncoupling effects is that the drugs induce a permeability to anions.

EXPERIMENTAL AND RESULTS - When mitochondria respiring in a lightly buffered salt medium are treated with 12.5 μM N,N' bis-dichloroacetyl,1,12-diaminodo-

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decane (C-12) respiration accelerates concomitant with an alkalization of the medium. Unlike strong uncouplers such as m-chlorocarbonylcyanidephenylhydrazone (CCP), Fig. 1 shows that this alkalization is transient and is followed by a period of relatively rapid acidification of the medium which returns to a base rate of acidification at approximately the same time that the rate of respiration slows to steady rate, suggesting the return to a new State 4-like condition. Once this new rate of respiration is established and the acidification of the medium returns to the original rate, the entire cycle can be repeated with essentially the same results. Atanaerobiosis there is an ex-

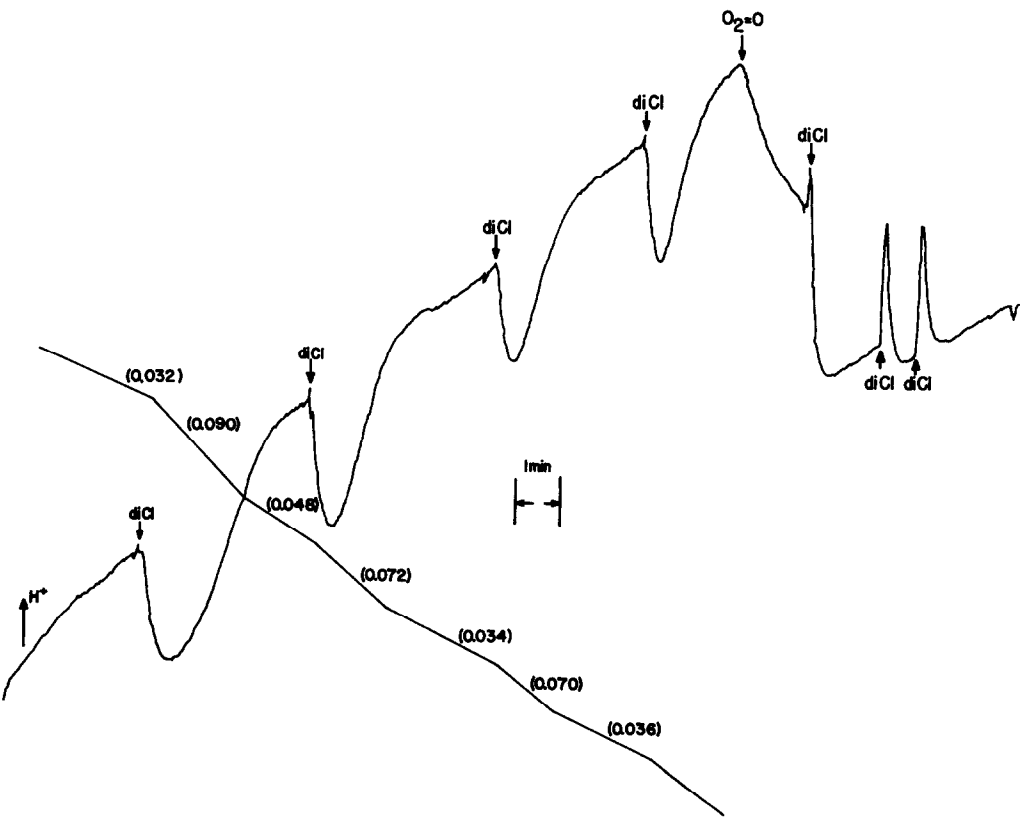

Figure 1 - The effect of the dichloroacetamide on pH and oxygen uptake in 100 mM KCl, 2 mM Tris Cl, pH 7.2. The substrate was succinate (2 mM). Drug (labeled d·Cl) was added in ethanol. Oxygen uptake in µatoms/min/mg is indicated along with the uptake trace measured with a Clark electrode. Total volume of the media was 8.0 ml and 5 mg mitochondrial protein was used. Mitochondria were prepared by the Nagarse method (4).

tensive alkalization of the medium which can be accelerated somewhat by a further addition of C-12 or CCP. Subsequent additions of C-12 after this point results in oxygen pulse-like pH changes resembling those obtained in a KCl-valinomycin medium or in the presence of a low level of uncoupler, due to oxygen dissolved in the ethanolic solution of drug.

Fig. 2 shows that this pH change is much more extensive in the absence of a respiratory substrate and is, in fact, as great or greater than that induced by uncoupling levels of CCP. Addition of succinate after the alkalization is complete results in rapid acidification after which the mitochondria again appear coupled since they respond to an addition of ADP and exhibit respiratory control and an ADP:O ratio of 1.5-1.7. When CCP is used under the same conditions there is little response to succinate or ADP (Fig. 2). In

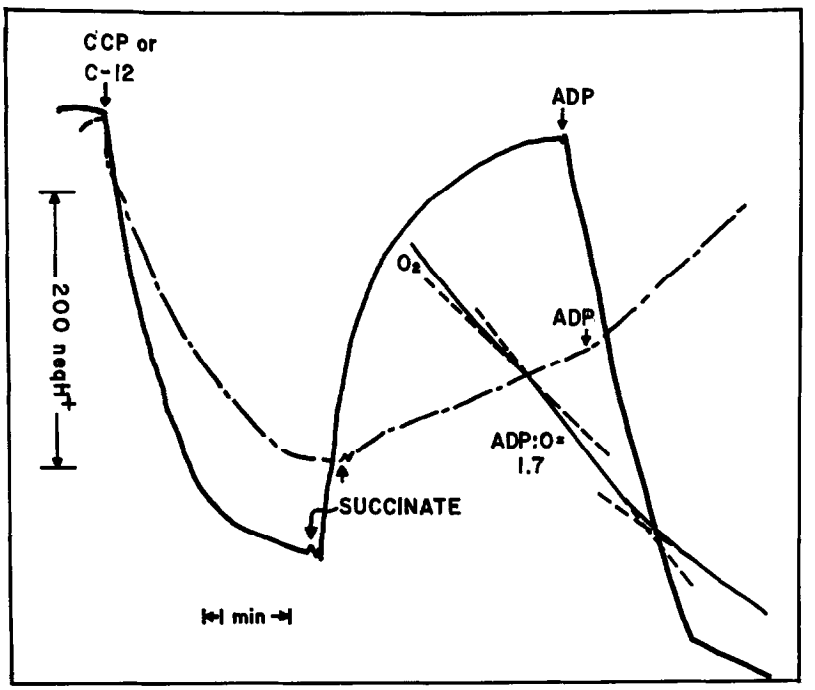

Figure 2 - Alkalization induced by the dichloroacetamide under passive conditions and succinate induced reversal of the pH transition. The medium was the same as that of Figure 1. The solid line is a trace of an experiment using the dichloroacetamide while the broken line is a comparison trace using CCP as the uncoupling agent. A recorder adjustment was necessary after the addition of succinate and ADP. The O_2 trace was obtained in the experiment using the dichloroacetamide.

experiments not shown, CCP added after the completion of the alkalization induced by C-12 produced no further change but did induce a typical alkalization if added after the succinate induced acidification had been completed.

The pH changes induced by C-12 seen in Fig. 1 and 2 could be due to protons entering the mitochondrion or due to hydroxide appearing in the medium. The experiments illustrated in Figs. 3 and 4 suggest that it is the anion which moves, probably at least initially, in exchange for an anion in the medium. Heart mitochondria are relatively impermeable to Cl^- at neutral pH (5, 6) and do not swell appreciably passively in KCl even in the presence of valinomycin. If the dichloroacetamide is added under these conditions a slow swelling occurs and chloride uptake under these conditions is about 180 n moles/mg. protein. This change is neither inhibited nor stimulated by uncoupling levels of CCP (Fig. 3b). In contrast to these results, C-12 has no marked effect on the energy-linked swelling induced by valinomycin (Fig. 3a) at a concentration which induces a maximal rate of respiration and a maximal pH change under passive conditions. CCP blocks the effect of this ionophore at

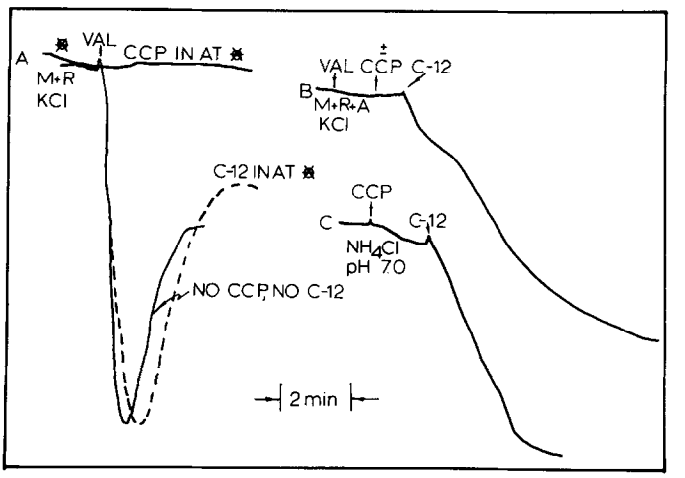

Figure 3 - The effect of the dichloroacetamide on active and passive swelling in KCl and passive swelling on NH_4Cl (100 mM pH 7.2). In trace A the mitochondria were respiring with succinate as substrate in the presence of rotenone. Valinomycin (0.25 µM) was added to induce swelling. Trace B is passive swelling in the same medium in the absence of substrate and with antimycin added as well as rotenone. Trace C is swelling in NH_4Cl in the presence of 0.4 µM CCP. Swelling was measured at 546 nM. Cl^- was determined as described by Hunter et. al. (7).

low levels of uncoupler (8). The inability of Cl to enter the mitochondrion is also reflected in the failure of mitochondria to swell extensively at neutral pH in NH_4Cl even in the presence of CCP under passive conditions. Figure 3c illustrates the ability of the dichloroacetamide to induce anion uptake under these conditions with a resultant swelling. Results similar to these were also obtained in NH_4Cl at elevated pH and in NH_4NO_3 at neutral pH with the only difference being that under these conditions the dichloroacetamide potentiates a fairly substantial uncoupler induced swelling.

Passive swelling in K^+ acetate is slow even in the presence of valinomycin. Under these conditions CCP induces a rapid swelling which may be due to the uncoupler acting as a proton conductor permitting the efflux of H^+ and thereby allowing the buildup of K^+ acetate in the interior (9). The dichloro-

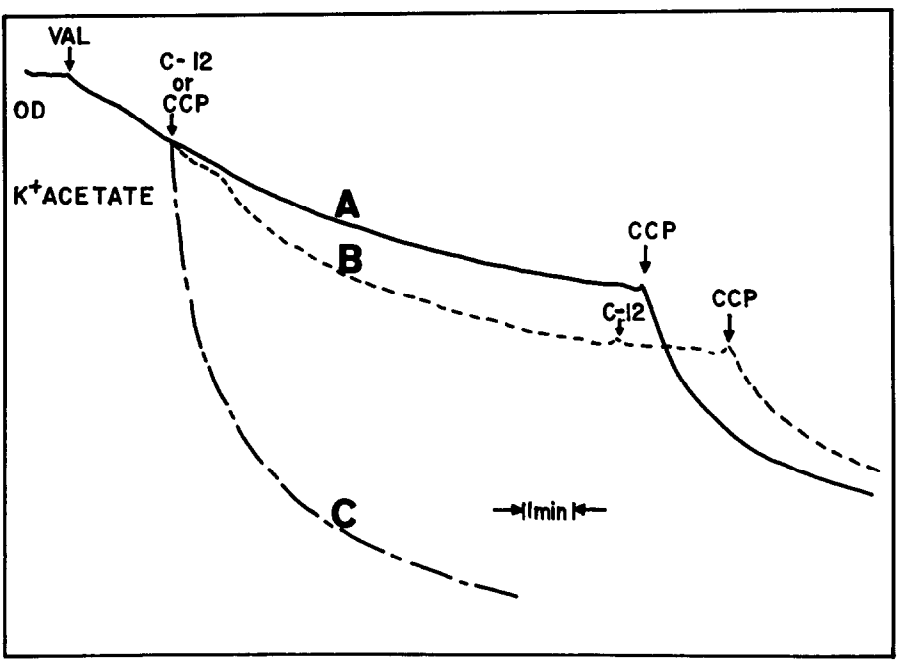

Figure 4 - Swelling in K^+ acetate pH 7.0 under passive conditions. CCP (0.4 μM) or the dichloroacetamide (12 μM) was added where indicated. The solid line (A) is a swelling trace of valinomycin treated mitochondria with no other additions until an addition of CCP after several minutes. The dashed line (B) is a similar trace but with the dichloroacetamide added where indicated followed by a second addition of this drug and then CCP. The broken line (C) is a trace of swelling induced by CCP following valinomycin addition with no other additions.

acetamide does potentiate swelling somewhat, but this potentiation is clearly much less than that induced by CCP (Fig. 4). Furthermore, addition of CCP after C-12 has been added results in a rapid rate of swelling while addition of a second pulse of C-12 has no effect on the rate of swelling.

DISCUSSION - N,N' bis-(dichloroacetyl) 1,12-diaminododecane, a homologue of the antispermatogenic agent N,N' bis-(dichloroacetyl) 1,8 diaminooctane possesses several properties which make it a potentially useful tool in the study of electron transport and energy conservation. Like disulfiram (10) and dipyridamole (11) the compound blocks pyridine nucleotide-linked oxidation with little effect on the oxidation of succinate (2). A transient permeability induced by uncouplers has been reported by Caswell and Pressman (12) who showed a reversible change in K^+ permeability in liver mitochondria. The data presented here show that this uncoupler acts cyclicly in buffered KCl probably by inducing a transient permeability to Cl^- and OH^- with little or no effect on K^+. Cyclic responses similar to these have been reported for thiophene derivatives by Schäfer and Büchel (13) who felt that these drugs were accumulated within the mitochondrion in an energy dependent reaction much like the uptake of Ca^{2+} (14). It seems more likely, based on the data presented here, that the more neutral dichloroacetamide uncouples by inducing anion permeability rather than by being the anion taken up. A similar effect was reported by Selwyn et. al. (15) who showed that trialkyl- and triphenyltin compounds induced Cl^- for hydroxide exchange and therefore swelling in KCl when valinomycin and an uncoupler were also present. The dichloroacetamide does not require the presence of an uncoupler in this system suggesting that its mechanism of action is different from the tin compounds. The latter can exist in the Cl^- and OH^- forms and may shuttle these anions in this way (15). Our present hypothesis as to the mechanism of action of the dichloroacetamide is that the drug combines with the mitochondrion causing a collapse of the trans-membrane pH gradient by inducing anion permeability. In KCl, this is

probably facilitated by a Cl^- for OH^- exchange since both anions appear to be permeable under these conditions. If a respiratory substrate is present, respiration is stimulated resulting inan acidification of the medium. Under passive conditions the pH change is complete as compared with the change which occurs at anaerobiosis or with the strong uncouplers and the mitochondria swell, provided a mechanism for cation entry is available eg. valinomycin in KCl or NH_3 penetration in NH_4Cl. Several possibilities for the cyclic response exist, including, active transport of the drug itself to some inert compartment or perhaps metabolism of the drug. A proposed mechanism will have to await the result of current investigations using labeled drug.

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