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Interacting Effects of Dibutylchloromethyltin Chloride, 2,3-Dimercaptopropanol, and Other Reagents on Mitochondrial Respiration and K^+ Flux[1]

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Abstract

The oxidative phosphorylation inhibitor DBCT (dibutylchloromethyltin chloride) inhibits state 3 respiration at a concentration less than that which stimulates K^+ flux into respiring rat liver mitochondria. Inhibition of ADP-stimulated respiration by DBCT can be reversed or blocked by the dithiol 2,3-dimercaptopropanol. The data are consistent with previous suggestions that DBCT may interact with the ATP synthase via reaction with a dithiol group. The stimulation of K^+ influx by DBCT is partially reversed by concentrations of 2-mercaptoethanol which fail to affect the inhibition of state 3 respiration by DBCT. The combination of DBCT plus 2,3-dimercaptopropanol inhibits mitochondrial K^+ influx. The inhibitory effect of dicyclohexylcarbodiimide on K^+ influx is not expressed in the presence of DBCT. Atractyloside has little effect on K^+ influx in the presence or absence of DBCT. The combination of DBCT plus uncoupler induces a net loss of endogenous K^+. Consideration is given to the alternative hypotheses that the acceleration of K^+ influx by DBCT may involve either a direct link to the energy transduction apparatus, or may occur via separate activation of a passive transport mechanism.

Key Words: Mitochondria; K^+ flux; respiration; dibutylchloromethyltin chloride; 2,3-dimercaptopropanol.

Introduction

Various trialkyltin compounds are known to bind with high affinity to mitochondria and inhibit oxidative phosphorylation (Aldrich *et al.*, 1977; Cain and Griffiths, 1977). The tin derivative DBCT is reported to react covalently with a constituent of the inner mitochondrial membrane (Cain *et*

[1]Abbreviations used: DBCT, dibutylchloromethyltin chloride; BAL, British Anti-Lewisite or 2,3-dimercaptopropanol; 2-ME, 2-mercaptoethanol; DCCD, dicyclohexylcarbodiimide; DNP, 2,4-dinitrophenol.

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al., 1977). The binding site for DBCT appears to be distinct from the site of interaction of DCCD, another inhibitor of the ATP synthase complex (Cain *et al.*, 1977). However, interaction between binding sites for DBCT and DCCD is observed (Kiehl and Hatefi, 1980; Partis *et al.*, 1980). Binding of triphenyltin to a low-molecular-weight proteolipid component of mitochondria has been reported (Dawson *et al.*, 1982).

The inhibitory effects of DBCT are reversed by treatment with the dithiol compounds dihydrolipoate, dihydrolipoamide, and dithiothreitol (Cain *et al.*, 1977; Stiggall *et al.*, 1979). On this basis it was suggested (Stiggall *et al.*, 1979) that DBCT may react with Coupling Factor B, an ATP synthase subunit which is known to interact with dithiol-selective reagents (Shankaran *et al.*, 1975; Joshi *et al.*, 1979; Joshi and Hughes, 1981). It should be noted that Factor B differs in solubility properties and size (Joshi and Sanadi, 1979; Sanadi, 1982) from the proteolipid component of mitochondrial membranes which is reported to bind triphenyltin (Dawson *et al.*, 1982).

Unidirectional K^+ flux into mitochondria is catalyzed by a mechanism which is saturable, respiration-dependent, and pH-dependent (Diwan and Tedeschi, 1975; Diwan and Lehrer, 1978; Jung *et al.*, 1977). The mechanism mediating K^+ efflux is also pH-dependent and respiration-dependent (Diwan and Tedeschi, 1975; Chavez *et al.*, 1977; Diwan, 1981). Swelling of mitochondria (Azzone *et al.*, 1978; Dordick *et al.*, 1980) or discharge of endogenous Mg^{2+} by the ionophore A23187 (Azzone *et al.*, 1978; Garlid, 1980; Jung *et al.*, 1981; Nakashima *et al.*, 1982) have been found to activate apparent net K^+/H^+ exchanges. Mercurials enhance rates of both entry and loss of K^+ (Jung *et al.*, 1977; Chavez *et al.*, 1977; Diwan *et al.*, 1977; Bogucka and Wojtczak, 1979). It has been suggested that K^+ influx and efflux may be mediated by separate mechanisms (Jung *et al.*, 1977).

Various inhibitors of oxidative phosphorylation, including DCCD, DBCT, and oligomycin, have been found to alter rates of respiration-dependent K^+ influx under some conditions (Gauthier and Diwan, 1979; Diwan, 1982; Jung *et al.*, 1980). Thus the possibility of a direct involvement of the ATP synthase in K^+ transport has been considered (Diwan, 1982). However, whether such reagents act separately on the K^+ transport mechanism or via interaction with the ATP synthase remains uncertain. An apparent inhibition of K^+ permeability by ADP in the presence of oligomycin has been interpreted as indicating a role of the adenine nucleotide translocase in K^+ transport (Panov *et al.*, 1980). Transmembrane K^+ movements accompany electrogenic ATP/ADP exchange under some conditions (LaNoue *et al.*, 1978). However, whether the link to adenine nucleotide translocation is direct or indirect is also unclear.

DBCT enhances the rate of respiration-dependent K^+ influx, measured by means of ^{42}K (Diwan, 1982). Since the stimulatory effect on K^+ influx of

the combined presence of DBCT plus the mercurial mersalyl is greater than the sum of the effects caused by each reagent alone, more than a single common site of action may be indicated (Diwan, 1982). The effects of some other alkyltin derivatives on mitochondrial permeability have been attributed to the ability of these reagents to catalyze Cl^-/OH^- exchange (Aldridge *et al.*, 1977; Bygrave *et al.*, 1978; Harris, 1978; Brierley *et al.*, 1978). However, the stimulation of K^+ influx by DBCT shows little dependence on Cl^- concentration (Diwan, 1982). DBCT has also been shown not to affect transmembrane pH shifts in plant mitochondria suspended in high Cl^- medium (Moore *et al.*, 1980).

Activation of K^+-dependent H^+ ejection from mitochondria by the dithiol-reactive reagents Cd^{2+} and phenylarsine oxide has been reported (Sanadi *et al.*, 1981). K^+ uptake, as evidenced by mitochondrial swelling, is found to correlate with Cd^{2+}-induced H^+ ejection (Rasheed and Sanadi, 1983). These effects of Cd^{2+} and phenylarsine oxide are blocked by treatment with a dithiol, BAL or dithiothreitol, but not by the monothiol, 2-ME (Sanadi *et al.*, 1981; Rasheed and Sanadi, 1983). On the basis of the differential effects of Cd^{2+} in the presence and absence of ruthenium red, which blocks Cd^{2+} uptake, Cd^{2+} is proposed to activate K^+ transport by binding to a site on the cytoplasmic surface of the inner mitochondrial membrane (Rasheed and Sanadi, 1983). This site appears to be distinct from a site on the matrix side of the membrane, possibly on Coupling Factor B, responsible for the uncoupling effects of Cd^{2+} (Rasheed and Sanadi, 1983). Phenylarsine oxide stimulates ^{42}K flux into respiring mitochondria (Srivastava and Diwan, 1983). This stimulatory effect of phenylarsine oxide is reversible by BAL and, to a lesser extent, by equivalent concentrations of 2-ME (Srivastava and Diwan, 1983).

The present studies have compared effects of the dithiol, BAL, and the monothiol, 2-ME, on the stimulation of K^+ influx and the inhibition of state 3 respiration by DBCT. The effects of some reagents which interact with the energy transduction apparatus on the stimulation of K^+ flux by DBCT have also been tested.

Materials and Methods

Rat liver mitochondria were isolated by standard procedures as in previous studies (e.g., Diwan *et al.*, 1979). The 0.25 M sucrose isolation medium was supplemented with 0.4 mM Tris EGTA in the initial stages of preparation. Mitochondrial respiration was monitored by means of a membrane-covered oxygen electrode connected to a potentiometric recorder. Respiration measurements were carried out at 20°C in medium containing 200 mM sucrose, 2.9 mM succinate, 1.9 mM K^+ (added as KOH), and 3.8

mM P_i (added as phosphoric acid), adjusted to pH 7.5 with Tris. Following recording of the state 4 rate, 0.3 mM Tris ADP was added to determine the state 3 respiration rate.

For K^+ flux measurements, mitochondria were incubated at 20°C in medium containing 200 mM sucrose, 8 mM succinate, and K^+ (added as KOH or included later with the ^{42}K, see figure legends), adjusted to pH 7.5 with Tris, plus trace amounts of ^3H_2O and ^{42}K, and, in some experiments, [^{14}C]sucrose. Sampling was by centrifugation through silicone (Harris and VanDam, 1968). Radioisotopes were assayed by liquid scintillation counting. Net K^+ levels were determined by atomic absorption spectroscopy. Mitochondrial contents of labeled and total K^+ were calculated from the data as in previous studies (e.g., Johnson and Pressman, 1969; Diwan *et al.*, 1979). Unidirectional K^+ influx rates were estimated from the change in mitochondrial content of labeled K^+ between an initial sample (0.75 min, except in experiment of Fig. 3 and Table IIA in which the initial sample was at 2 min) and a sample taken after 7 or 8 min of incubation.

Mitochondrial protein was assayed by the Biuret procedure (Layne, 1957). Radioisotopes were obtained from New England Nuclear. Silicone (SF1154 or SF1230) was a gift of the General Electric Co. BAL was obtained from Aldrich Chemical Company. 2-ME was obtained from Eastman Organic Chemicals and from Aldrich Chemical Company. Atractyloside and oligomycin were obtained from Sigma Chemical Company. DBCT was supplied by Dr. D. E. Griffiths.

Results

ADP-Stimulated Respiration

The data in Fig. 1 show the inhibitory effect of DBCT on state 3 respiration as a function of DBCT concentration. Half maximal inhibition of state 3 respiration is observed at approximately 0.9 μmol DBCT per gram of protein (1.4 μM). At concentrations of DBCT above about 4 μmol per gram of protein the rate of respiration in the presence of ADP approaches the state 4 rate. In contrast, DBCT has little effect on state 4 respiration, causing only a slight stimulation at high concentrations (see below).

The inhibitory effect of DBCT on state 3 respiration may be prevented or reversed by treatment with the dithiol BAL. The concentration of BAL required to block the effect of DBCT depends on whether the DBCT is allowed to interact with the BAL prior to reaction with the mitochondria. Figure 2A shows blockage of the DBCT inhibition of state 3 respiration by varied concentrations of BAL included with DBCT in the medium prior to addition of the mitochondria. Figure 2B shows reversal of the effect of DBCT

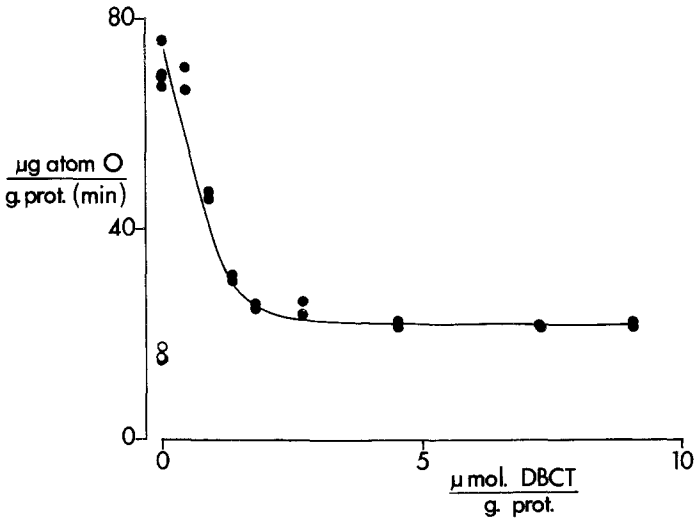

Fig. 1. Dependence of mitochondrial respiration on DBCT concentration. The mitochondrial protein concentration was 1.6 mg/ml. The respiration rate, in units of μg-atoms O per gram of protein per minute, is plotted against the concentration of DBCT in the medium, in units of μmol per gram of protein. The line is drawn arbitrarily. Symbols: (●) state 3 respiration values; (○) state 4 respiration values.

by addition of varied concentrations of BAL 1 min after addition of mitochondria to the medium containing DBCT. Consistently a higher concentration of BAL is required for a given extent of relief of the inhibitory effect of DBCT if the BAL is added after reaction of DBCT with the mitochondria. Other experiments have shown that BAL when added alone has no significant effect on state 3 respiration under the conditions of these experiments (See, e.g., Table I).

BAL has little effect on state 4 respiration in the presence of DBCT. For example, in the experiment of Fig. 2A, the observed rates of state 4 respiration were 18.9 ± 0.6 μg-atoms O per gram of protein per minute for control mitochondria (mean of three determinations ± SD), compared to values of 22.2 and 24.6 μg-atoms O per gram of protein per minute in the presence of 4.5 μmol DBCT per gram of protein (7.1 μM). The presence of 40 μmol BAL per gram of protein (64 μM) in the medium along with the DBCT resulted in state 4 respiration rates of 20.9 and 21.2 μg-atoms O per gram of protein per minute. In the experiment of Fig. 2B, state 4 respiration values were 20.4 ± 1.4 (mean of five determinations ± SD) for control mitochondria and 26.9 ± 2.0 (mean of three determinations ± SD) μg-atoms O per gram of protein per minute in the presence of 4.5 μmol DBCT per gram of protein (7.9 μM). Addition of 84 μmol BAL per gram of protein (145 μM) 1 min after the

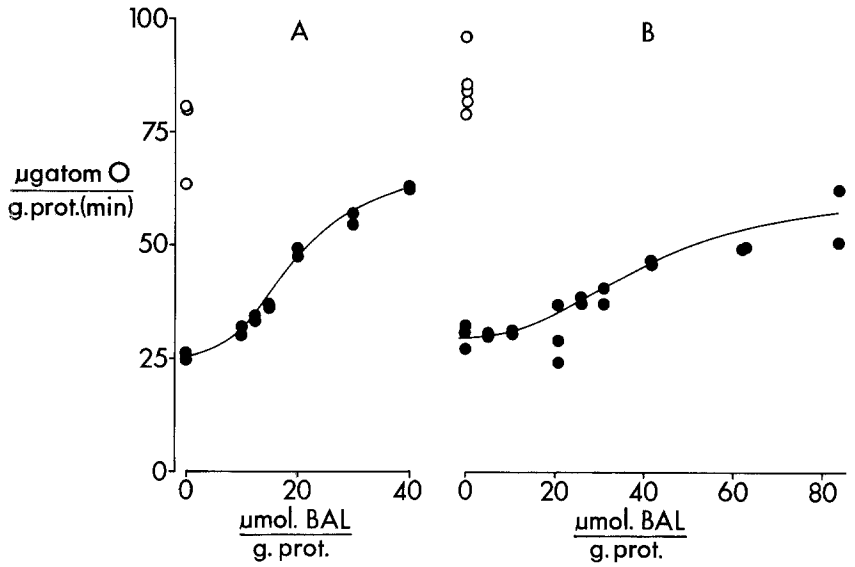

Fig. 2. Blockage or reversal by BAL of the inhibition of state 3 respiration by DBCT. In experiment A, the mitochondrial protein concentration was 1.6 mg/ml. BAL at the concentrations indicated on the abscissa was present in the medium prior to addition of the mitochondria. In experiment B, the mitochondria (protein concentration 1.7 mg/ml) were added to the medium at zero time. After 1 min, BAL was added at the concentrations indicated on the abscissa. ADP was added 2 min after addition of the mitochondria. Symbols: (●) the medium included 4.5 μmol DBCT per gram of protein (7.1 or 7.9 μM); (○) the medium contained no DBCT.

Table I. Comparison of Effects of BAL and 2-ME on the Inhibition of State 3 Respiration by DBCT[a]

Other additions		State 3 respiration (μg-atoms O/g protein · min)	
at zero time	at 1 min	− DBCT	+ DBCT
—	—	95	29
—	—	99	29
—	—	98	28
BAL	—	97	62
BAL	—	97	57
—	BAL	99	60
—	BAL	—	58
2-ME	—	101	30
2-ME	—	103	29
—	2-ME	—	30
—	2-ME	—	28

[a]The mitochondrial protein concentration was 2.0 mg/ml. DBCT when included was at 4.5 μmol/g protein (8.9 μM), in the medium from zero time. When present from zero time, BAL was at 27 μmol/g protein (55 μM) and 2-ME at 55 μmol/g protein (109 μM). When added 1 min after the mitochondria, BAL was at 55 μmol/g protein (109 μM) and 2-ME at 110 μmol/g protein (218 μM). ADP was added 2 min after the mitochondria.

DBCT resulted in state 4 respiration rates of 20.7 and 21.3 μg-atoms O per gram of protein per minute. Thus the only apparent effect of BAL is reversal of the slight uncoupling effect of DBCT. In other experiments, addition of BAL alone was found to have no effect on state 4 respiration.

Table I compares the ability of BAL and 2-ME to block or reverse the inhibition of state 3 respiration by DBCT. The effect of 2-ME was tested at twice the concentration of BAL in each case, so that an equivalent concentration of sulfhydryl groups could be compared. Unlike BAL, 2-ME is found not to block or reverse the inhibitory effect of DBCT on state 3 respiration. The data of Table I and other experiments indicate that neither BAL nor 2-ME significantly affects state 3 respiration when added alone under these conditions. It should be recalled that the inhibitory effects of BAL on electron transfer activities that have been described (Van Ark *et al.*, 1981; Zhu *et al.*, 1982) require prolonged treatment with BAL at much higher concentrations than those used in the present studies.

Mitochondrial K^+ Transport

The ability of BAL and 2-ME to reverse the stimulation by DBCT of K^+ flux into respiring mitochondria is examined in the experiment depicted in

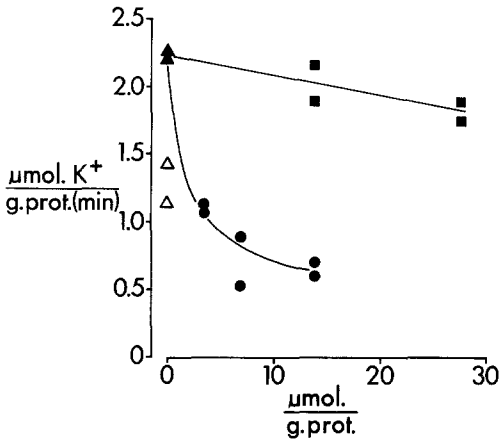

Fig. 3. Effect of BAL and 2-ME on K^+ flux into mitochondria treated with DBCT. The mitochondrial protein concentration was 5.2 mg/ml. The K^+ concentration in the medium was 2.0 mM. DBCT when present was included in the medium from zero time at 6.8 μmol/g protein or 35 μM. BAL or 2-ME when present were added, at the concentrations shown on the abscissa, 1 min after addition of the mitochondria. Reagents used: (△) control; (▲) DBCT; (■) DBCT + 2-ME; (●) DBCT + BAL.

Table II. Effects of Some Metabolic Inhibitors on K^+ Influx in the Presence of DBCT

Experiment	Other additions	K^+ influx (μmol K^+/g protein · min)	
		− DBCT	+ DBCT
A	None	1.06 ± 0.06	1.64 ± 0.14
	BAL	1.14 ± 0.08	0.56 ± 0.11
	2-ME	1.07 ± 0.05	1.39 ± 0.07
B	None	1.50 ± 0.08	2.57 ± 0.20
	Oligomycin	1.60, 1.43	2.46, 2.67
C	None	1.88 ± 0.11	3.87 ± 0.19
	DCCD	1.48 ± 0.14	4.67 ± 1.23
D	None	0.92 ± 0.02	1.95 ± 0.05
	Atractyloside	0.95 ± 0.07	1.79 ± 0.08

[a] In experiments A, B, C, and D the mitochondrial protein concentrations were 5.0, 5.4, 5.1, and 3.9 mg/ml; the K^+ concentration in the medium was 1.5, 2.8, 4.3, and 2.3 mM; and DBCT when present in the medium was at 6.5, 6.1, 7.8, and 6.7 μmol per gram of protein, corresponding to molar concentrations of 33, 33, 40, and 26 μM respectivley. In A, BAL (14 μmol per gram protein or 70 μM) or 2-ME (28 μmol per gram protein or 140 μM) when included was added 1 min after addition of the mitochondria to the medium. In B, oligomycin when present in the medium was at 0.47 μg/mg protein. In C, the DCCD-treated mitochondria were preincubated at 0°C for at least 40 min with 31 μmol DCCD per gram protein. In D, atractyloside when present in the medium was at a concentration of 10 μM. Means of three determinations ± standard deviations, or individual values are listed.

Fig. 3 and in experiment A of Table II. 2-ME consistently causes some reversal of the stimulatory effect of DBCT on K^+ influx. When added to mitochondria pretreated with DBCT, BAL has an inhibitory effect on K^+ influx. The data in Fig. 3 could be interpreted as indicating reversal of the effect of DBCT by the lowest concentration of BAL tested (3.5 μmol/g protein or about half the DBCT concentration). However, at BAL concentrations equal to or even slightly less than the concentration of DBCT, the inhibitory effect is evident. BAL, when added alone, has little effect on K^+ influx under the conditions of these experiments, as shown in Table IIA. Other experiments (data not shown) have indicated that the combination of DBCT plus BAL also inhibits K^+ influx when the BAL is allowed to interact with the DBCT in the medium prior to addition of the mitochondria.

Experiments C, D, and E of Table II test for effects on K^+ influx of adding other inhibitors of adenine nucleotide metabolism along with the DBCT. Oligomycin fails to affect K^+ influx in the presence of DBCT (experiment B). DCCD pretreatment alone inhibits K^+ influx, consistent with earlier studies (Gauthier and Diwan, 1979), as shown in experiment C. However, in the presence of DBCT, DCCD pretreatment consistently fails to have any inhibitory effect. Thus the DBCT-stimulated K^+ influx rate is

Table III. Effects of DNP on K^+ Flux in the Presence of DBCT or Oligomycin[a]

Additions	K^+ influx (μmol K^+/g protein · min)	7 min K^+ content (μmol K^+/g protein)
None	1.17 ± 0.08	87 ± 6
DNP	0.14 ± 0.03	78 ± 5
DBCT	2.45 ± 0.22	96 ± 5
DNP + DBCT	(0.59 ± 0.03)	22 ± 1
Oligomycin	1.01 ± 0.12	85 ± 4
DNP + Oligomycin	0.18 ± 0.03	71 ± 2

[a]The mitochondrial protein concentration was 4.6 mg/ml. The K^+ concentration in the medium was 2.1 mM. When present in the medium, DNP was at 60 μM, DBCT was at 6.7 μmol/g protein or 31μM, and oligomycin was at 0.55 μg/mg protein. The values shown are means of three determinations ± standard deviations.

equivalent in the presence and absence of DCCD. Atractyloside, at a concentration sufficient to block the adenine nucleotide translocase (Duee and Vignais, 1969), has little effect on K^+ influx, causing only a very slight inhibition in the presence of DBCT (experiment D). Separate measurements have confirmed that the sample of atractyloside tested was capable of blocking ADP-stimulated respiration under approximately similar conditions (data not shown).

Table III examines the effect on mitochondrial K^+ flux of the combined presence of DBCT and the uncoupler DNP. Addition of the uncoupler alone markedly inhibits K^+ flux into the respiring mitochondria, while causing some net loss of endogenous K^+, as previously reported (e.g., Diwan and Tedeschi, 1975). When both DNP and DBCT are present in the medium, a greatly stimulated net efflux of K^+ occurs, as indicated by the difference in the net K^+ contents at the end of the 7-min incubations. Under such conditions in which a large net K^+ flux is occurring, the ^{42}K uptake measurements do not accurately represent unidirectional K^+ influx rates. In contrast to the effect of DBCT, the oxidative phosphorylation inhibitor oligomycin has little effect on unidirectional or net K^+ flux rates in the presence of DNP.

Discussion

The observed inhibition of ADP-stimulated respiration by DBCT is consistent with earlier reported effects of DBCT on oxidative phosphorylation and oligomycin-sensitive ATPase activities (Cain *et al.*, 1977). The ability of BAL, but not 2-ME, to reverse the inhibition of state 3 respiration by DBCT supports the conclusion that DBCT interacts with the ATP synthase via a dithiol group. There remains the question of whether a common site of interaction is involved in the effects of DBCT on energy transduction and on K^+ transport.

Some evidence suggests that the sites of interaction of DBCT with the ATP synthase and with the K^+ transport mechanism may be at least partially distinct. The concentration of DBCT required to block ADP-stimulated respiration is less than that required to stimulate K^+ influx. DBCT was previously observed to stimulate K^+ influx only at concentrations in excess of about 5 μmol per gram of protein, which was in the range of 24–28 μM under the conditions of those experiments (Diwan, 1982). In the present studies, some inhibition of state 3 respiration by DBCT has been observed at concentrations as low as 0.9–1.5 μmol per gram of protein, corresponding to 1.4–3.6 μM. (The higher corresponding micromolar concentrations in the K^+ transport studies are attributable to the technical requirement for higher concentrations of mitochondrial protein for the ^{42}K influx measurements.)

Higher concentrations of DBCT (6.5–6.8 μmol/g protein or 33–40 μM) were used in the present ^{42}K flux studies focusing on effects of 2-ME in the presence of DBCT, compared to the concentrations of DBCT (e.g., 4.5 μmol/g protein or 8.9 μM) used in the similar respiratory studies. Nevertheless, 2-ME was found to partially reverse the effects of DBCT on K^+ influx at a concentration (28 μmol/g protein or 140 μM) less than that found not to reverse the effect of DBCT on state 3 respiration (110 μmol/g protein or 218 μM). These findings suggest different sites of action of DBCT in affecting oxidative phosphorylation and K^+ transport.

The inhibitory effect of low concentrations of BAL in the presence of DBCT was seen only with K^+ transport. The results suggest that BAL affects K^+ transport in some way other than by simply antagonizing DBCT binding. Since BAL alone has no effect, some type of synergistic action of DBCT plus BAL is indicated. An effect of low concentrations of BAL in potentiating effects of arsenite has been interpreted in terms of a role of BAL in aiding penetration of the arsenite to a site of action (Fluharty and Sanadi, 1961). Whether BAL might have a similar role in facilitating access of the more lipid-soluble DBCT to some membrane site is uncertain.

The inhibitory effect of DCCD on K^+ influx is not expressed in the presence of DBCT. As pointed out earlier, interaction between binding sites for DBCT and DCCD on the ATP synthase has been proposed (Kiehl and Hatefi, 1980; Partis *et al.*, 1980). However, it has also been suggested that DCCD may affect K^+ transport via reaction with some site distinct from the DCCD-binding ATP synthase subunit (Jung *et al.*, 1980). While the present results indicate alteration by DBCT of effects of DCCD reaction with the site relevant to K^+ transport, the results do not rule out the possibility that this site may not be on the ATP synthase complex.

The failure of atractyloside to significantly affect K^+ influx rates either in the presence or absence of DBCT is inconsistent with a direct role of the

adenine nucleotide translocase in respiration-dependent K^+ transport, at least under the conditions of these experiments.

DBCT and the uncoupler DNP interact to produce a marked activation of K^+ efflux. In fact the process of K^+ transport in the presence of DBCT is similar to that in the absence of DBCT in that both K^+ influx and efflux rates are reduced in the presence of respiratory inhibitors, while K^+ efflux is stimulated in the presence of uncoupler (data of present studies; Diwan and Tedeschi, 1975; Diwan, 1982). Under the conditions of the ^{42}K flux experiments, the K^+ concentration gradient is such that there is a much higher concentration of K^+ in the mitochondrial matrix (approximately 100 mM, see Diwan and Tedeschi, 1975) than in the medium (e.g., 2.1 mM in the experiment of Table III). If one assumes enhancement of activity of a passive K^+ uniport mechanism by DBCT, discharge of the K^+ concentration gradient in the presence of uncoupler might be expected, since the contribution of a respiration-generated electrical gradient as a driving force for K^+ flux would be diminished by uncoupler-mediated H^+ entry (Nicholls, 1974). Such a discharge of K^+ is observed in the presence of valinomycin plus uncoupler (Pressman *et al.,* 1967). Alternatively, acceleration of K^+ efflux through reversal of an activated energy-linked pump mechanism, driven by uncoupler-induced dissipation of an energized intermediate, could be postulated (Diwan and Tedeschi, 1975).

Acknowledgments

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