Journal of Bioenergetics and Biomembranes, Vol. 18, No. 2, 1986

Stimulation of K^+ Flux into Mitochondria by Phenylarsine Oxide[1]

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Received October 15, 1985; revised December 9, 1985

Abstract

The dithiol-reactive reagent phenylarsine oxide causes a pH-dependent stimulation of unidirectional K^+ flux into respiring rat liver mitochondria. This stimulation is diminished by subsequent addition of either the dithiol 2,3-dimercaptopropanol or the monothiol 2-mercaptoethanol. In contrast, uncoupling by phenylarsine oxide is reversed by 2,3-dimercaptopropanol but not by 2-mercaptoethanol. The data suggest separate sites of interaction of phenylarsine oxide with mechanisms of K^+ entry and ATP synthesis. Stimulatory effects of mersalyl and phenylarsine oxide on K^+ influx are not additive. Thus PheASO and mersalyl may affect K^+ influx at a common site. Pretreatment of the mitochondria with DCCD, which inhibits K^+ influx, fails to alter sensitivity to PheAsO or mersalyl. Thus the DCCD binding site associated with the K^+ influx mechanism appears to be separate from and independent of the sulfhydryl group(s) which mediate stimulation of K^+ influx by PheAsO and mersalyl.

PheAsO, like mersalyl, also increases the rate of unidirectional K^+ efflux from respiring mitochondria. The combined presence of PheAsO plus mersalyl causes a greater stimulation of K^+ efflux than is observed with either reagent alone.

Key Words: K^+ transport; mitochondria; mersalyl; phenylarsine oxide; dithiol; monothiol.

Introduction

Unidirectional K^+ flux into mitochondria occurs via a mechanism which is saturable, respiration dependent, and pH dependent (Diwan and Tedeschi,

[1] Abbreviations used: BAL, British AntiLewisite or 2,3-dimercaptopropanol; DCCD, dicyclohexylcarbodiimide; DBCT, dibutylchloromethyltin chloride; 2-ME, 2-mercaptoethanol; PheAsO, phenylarsine oxide.

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0145-479X/86/0400-0123$05.00/0

1975; Diwan, 1977; Jung *et al.*, 1977; Diwan and Lehrer, 1978); and subject to inhibition by the K^+ analogs Tl^+ and Ba^{2+} (Diwan, 1977; Diwan, 1985). The mechanism limiting K^+ influx appears to be localised in the inner mitochondrial membrane, since removal of the outer membrane has little effect on the kinetics of K^+ influx (Chang and Diwan, 1982).

The influx of K^+ is subject to inhibition by DCCD (Gauthier and Diwan, 1979; Jung *et al.*, 1980). Pretreatment of mitochondria with DCCD increases the apparent K_m for K^+ of the influx mechanism (Gauthier and Diwan, 1979). DCCD reacts particularly with carboxylic acid groups of proteins, but under some conditions can also react with amines, alcohols, and thiol groups (Kurzer and Douraghi-Zadeh, 1967; Linnett and Beechey, 1979). Inhibition of proton translocation through the mitochondrial ATP synthase and cytochrome oxidase complexes has been attributed to modification of carboxylic acid groups of specific glutamate residues (Sebald and Hoppe, 1981; Prochaska *et al.*, 1981).

The process of K^+ efflux is also pH dependent and respiration dependent (Diwan and Tedechi, 1975; Chavez *et al.*, 1977; Diwan, 1981). Consistent with the chemiosmotic theory, it has been proposed that a passive uniport mediates electrophoretic K^+ entry, while K^+ efflux occurs via a separate K^+/H^+ antiport pathway (Mitchell and Moyle, 1969; Chavez *et al.*, 1977; Jung *et al.*, 1977). Such a model provides an explanation for the observations (Diwan and Tedeschi, 1975; Diwan *et al.*, 1979; Skulskii *et al.*, 1983) that both influx and efflux of K^+ and other cations are respiration dependent. The measured pH dependence of K^+ efflux does not support a passive K^+/H^+ antiport mechanism (Diwan, 1981); however, secondary effects of pH cannot be ruled out. It has been pointed out that the absence of evidence for net H^+ fluxes linked to K^+ movements leaves the existence of a K^+/H^+ antiport unproven (Brierley *et al.*, 1984).

Mercurial sulfhydryl reagents enhance rates of both entry and loss of K^+ (Chavez *et al.*, 1977; Jung *et al.*, 1977; Diwan *et al.*, 1977; Bogucka and Wojtczak, 1979). How mercurials activate mitochondrial K^+ transport is unclear. A suggestion that mercurials affect K^+ permeability by discharging endogenous Mg^{2+} (Bogucka and Wojtczak, 1979) is not supported by measurements of mitochondrial Mg^{++} (Diwan *et al.*, 1980) or by the sensitivity to mersalyl of K^+ flux into divalent cation depleted mitochondria (Jung *et al.*, 1981).

However, discharge of mitochondrial Mg^{2+} by treatment with the ionophore A23187 does appear to stimulate net K^+ fluxes which have been attributed to activity of the postulated K^+/H^+ antiporter (Azzone *et al.*, 1978; Garlid, 1980; Jung *et al.*, 1981; Nakashima *et al.*, 1982). The physiological significance of a permeability pathway which is activated by extreme conditions of Mg^{2+} depletion has been questioned (Jung *et al.*, 1984).

Inhibition of the apparent K^+/H^+ exchanger by DCCD is observed only if mitochondria are depleted of Mg^{2+} prior to DCCD treatment (Martin *et al.*, 1984). In contrast, the K^+ efflux pathway of mitochondria containing endogenous Mg^{2+} shows little sensitivity to DCCD (Jung *et al.*, 1980). An 82,000 dalton protein which may have a role in mediating K^+/H^+ exchange has been identified on the basis of the Mg^{2+} sensitivity of its reactivity with [^{14}C]DCCD (Martin *et al.*, 1984).

PheAsO, like other arsenicals and Cd^{2+}, uncouples oxidative phosphorylation (Fluharty and Sanadi, 1960, 1962, 1963; Sanadi *et al.*, 1981). The uncoupling by arsenicals is reversed by an excess of a dithiol, such as BAL, but is unaffected or potentiated by addition of a monothiol, such as 2-ME; thus involvement of an essential dithiol in energy coupling was postulated (Fluharty and Sanadi, 1960, 1962, 1963). Coupling factor B, a subunit of the ATP synthase, contains a PheAsO-sensitive dithiol site (Stiggall *et al.*, 1979; Joshi and Hughes, 1981; Sanadi, 1982; Yagi and Hatefi, 1984). This site appears to be inaccessible to membrane-impermeable mercurials in intact mitochondria (Sanadi, 1982).

PheAsO and Cd^{2+} cause K^+-dependent H^+ ejection from respiring mitochondria, which is followed by decay of the pH gradient as uncoupling occurs (Sanadi *et al.*, 1981; Sanadi, 1982; Rasheed *et al.*, 1984). ^{42}K uptake measurements have shown that Cd^{2+}, during the initial time period, enhances the rate of undirectional K^+ influx (Rasheed *et al.*, 1984). The mercurials mersalyl and *p*-hydroxymercuribenzoate induce H^+ ejection in the presence of K^+, but fail to subsequently collapse the pH gradient (Sanadi *et al.*, 1981; Sanadi, 1982). It has been suggested that mercurials act via reaction with an outward-facing site distinct from the dithiol of coupling factor B (Sanadi, 1982).

The oxidative phosphorylation inhibitor DBCT also stimulates unidirectional K^+ flux into respiring mitochondria (Diwan, 1982; Diwan *et al.*, 1983). Inhibition of oxidative phosphorylation by the tin derivative DBCT is reversible by dithiols, but not by monothiols (Cain *et al.*, 1977; Stiggall *et al.*, 1979; Diwan *et al.*, 1983). Thus DBCT may be dithiol reactive. However, inhibition of oxidative phosphorylation by another tin compound, triphenyltin chloride, was found to be sensitive to the monothiol 2-ME (Yagi and Hatefi, 1984). The stimulation of K^+ influx in the combined presence of DBCT plus mersalyl exceeds the sum of effects of each reagent alone, suggesting more than a single common site of action (Diwan, 1982). The monothiol 2-ME partially blocks the stimulation of K^+ influx by DBCT (Diwan *et al.*, 1983). Based on differing sensitivities to thiol reagents, it has been suggested that DBCT affects K^+ influx at some site other than the group responsible for inhibition of oxidative phosphorylation by DBCT (Diwan *et al.*, 1983).

The present studies have examined whether PheAsO stimulates mitochondrial K^+ influx as postulated by Sanadi and co-workers. Experiments have been aimed at comparing properties of thiol groups responsible for effects of PheAsO on K^+ influx and on energy transduction. Relationships among sites mediating effects on K^+ influx of PheAsO, mersalyl, and DCCD have been examined.

Materials and Methods

Rat liver mitochondria were isolated by standard procedures. 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. For the K^+ flux measurements, mitochondria (5–8 mg protein per ml) were incubated at 20°C in media (unless specified otherwise) containing 200 mM sucrose, 8 mM succinic acid, 1.3–3.2 mM K^+ (added as KOH and as carrier with the ^{42}K), ^{42}K (approximately 0.6 μCi/ml), ^3H_2O (approximately 3 μCi/ml), and in some experiments [^{14}C]sucrose (approximately 0.8 μCi/ml), with the pH adjusted to 7.5 with Tris. At timed intervals, mitochondrial samples were separated from incubation media by rapid centrifugation through silicone (Harris and VanDam, 1968). ^{42}K counts were assayed by liquid scintillation counting of the Cerenkov radiation in aqueous dilutions of mitochondrial and supernatant samples, and the counts were corrected for decay. Following decay of the ^{42}K, total K^+ levels were assayed by atomic absorption spectroscopy, and ^3H and ^{14}C were counted using a standard liquid scintillation cocktail. Protein was measured by the biuret technique (Layne, 1957). Fluid compartments, mitochondrial contents of total and labeled K^+, and unidirectional K^+ influx and efflux rates were calculated as in earlier studies (Johnson and Pressman, 1969; Diwan *et al.*, 1979).

Radioisotopes were obtained from New England Nuclear. Silicone SF1230 was a generous gift from the General Electric Co. PheAsO, BAL, and 2-ME were obtained from Aldrich Chemical Co., DCCD and mersalyl were from Sigma Chemical Co.

Results

Figura 1A shows the time course of ^{42}K uptake by mitochondria suspended in a medium at pH 7.5 containing succinate as respiratory substrate. PheAsO increases the unidirectional K^+ influx rate, corresponding to the slope of the uptake curve, without appreciably affecting the extrapolated zero

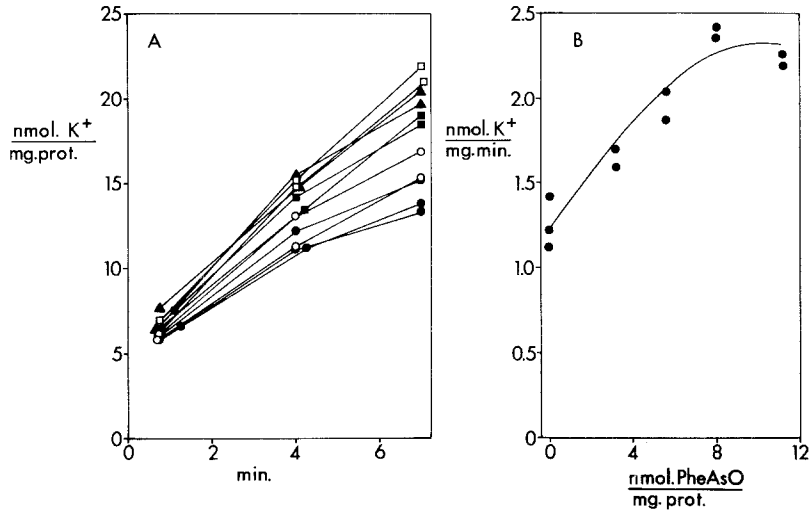

Fig. 1. Stimulation of K^+ influx by PheAsO. (A) Mitochondrial content of labeled K^+, in units of nmol per mg protein, is plotted against the incubation time in minutes. Values are corrected for contaminating external K^+, estimated from the product of the [^{14}C]sucrose distribution space and the supernatant K^+ concentration. PheAsO concentrations, in units of nmol per mg protein: ●, 0; ○, 3.2; ■, 5.6; □, 8.0; ▲, 11.2. (B) Unidirectional K^+ influx rates, determined from the change in labeled K^+ content between 0.75 and 7 min samples (data of Fig. 1A), in units of nmol K^+ per mg protein per minute, is plotted against PheAsO concentration, in units of nmol per mg protein.

time ^{42}K uptake. The rapid ^{42}K binding which occurs before the initial sample is taken has been attributed to adsorption (Diwan and Lehrer, 1978). During the 7-min incubations, under most conditions studied the turnover of endogenous K^+ is small relative to the total initial K^+ content, which averaged 93 nmol per mg protein for the experiment of Fig. 1 (see also data of Table I). Therefore efflux of the ^{42}K may be considered negligible. The ^{42}K uptake remains an approximately, though not perfectly, linear function of incubation time in the presence of PheAsO, as has been observed previously for control samples (e.g., Diwan and Lehrer, 1978). Thus the isotope uptake reflects an initial rate of K^+ entry. Within the time resolution of the measurements, the stimulation by PheAsO exhibits no time lag such as precedes uncoupling effects of arsenicals (Fluharty and Sanadi, 1960; Sanadi *et al.*, 1981). The leveling off of the K^+ influx rate above about 8 nmol PheAsO per mg protein shown in Fig. 1B relates to a consistent tendency toward decreased ^{42}K uptake at later times with the higher PheAsO concentrations (Fig. 1A). In the experiment of Fig. 1, atomic absorption measurements showed the mitochondrial K^+ content to be essentially constant, except that the 7-min samples in the presence of 11.4 nmol PheAsO per mg protein showed a

Table I. Effect of PheAsO and Mersalyl on K^+ Influx and Efflux at pH 7 and pH 8[a]

Additions	K^+ flux: (nmol K^+/mg protein per min)			
	K^+ influx		K^+ efflux	
	pH 7	pH 8	pH 7	pH 8
None	1.04 ± 0.33 (6)	1.57 ± 0.03 (6)	1.3 ± 0.5 (6)	2.3 ± 0.7 (6)
PheAsO	1.79 ± 0.10 (4)	3.83 ± 0.22 (4)	2.7 ± 0.6 (4)	3.7 ± 0.5 (4)
Mersalyl	2.04 ± 0.17 (4)	4.43 ± 0.16 (4)	1.7 ± 0.3 (4)	3.9 ± 0.6 (4)
Phe. and Mer.	1.47 ± 0.26 (4)	5.70 ± 0.09 (4)	7.4 ± 0.8 (4)	7.2 ± 0.2 (4)

[a]The medium included 200 mM sucrose, 8 mM succinate, 30 mM Tris, and 3.2 mM K^+, adjusted to pH 7.0 or 8.0 with HCl. PheAsO (10 nmol/mg protein or 50 μM) and mersalyl (150 μM) when included were in the medium from zero time. Unidirectional K^+ influx rates were determined from the change in labeled K^+ content between samples taken after 0.5 and 7 min of incubation. Net K^+ fluxes were determined from the change in total K^+ over the same time period. Unidirectional K^+ efflux rates are the differences between influx and net flux rates. Values are pooled from two experiments, with half of the samples for each condition being from each experiment. Values are means ± S.D. (number of determinations). The average 0.5 min total K^+ content, corrected for K^+ in the sucrose-penetrable space, was estimated to be 88 nmol/mg protein at pH 7 and 93 nmol/mg at pH 8.

significantly decreased total K^+ content of 75–80 nmol per mg protein compared to control values of 88–97 nmol per mg protein. The mitochondria otherwise remain close to steady state with respect to total K^+ content, because the stimulation of K^+ influx by PheAsO is accompanied by Stimulation of K^+ efflux (see also Table I).

Table I compares effects of PheAsO on unidirectional K^+ influx and efflux rates at pH 7 and at pH 8. The pH dependence of control influx and efflux rates is similar to that reported earlier (Chavez *et al.*, 1977; Diwan and Lehrer, 1978; Diwan, 1981). The stimulation of K^+ influx by PheAsO is greater at pH 8 than at pH 7. The stimulation of K^+ influx by mersalyl exhibits a similar pH dependence. It should be recalled that the pH dependence of the effect of mersalyl may be partially attributable (Diwan *et al.*, 1977) to inhibition of phosphate/hydroxyl exchange (Meijer *et al.*, 1970). The combined presence of mersalyl plus PheAsO at pH 8 causes only a slightly greater stimulation of K^+ influx than the presence of mersalyl alone.

The data of Table I also show stimulation of K^+ efflux by PheAsO. In contrast to the absence of a synergistic effect on K^+ influx, the combined presence of PheAsO plus mersalyl markedly enhances the rate of K^+ efflux, both at pH 7 and pH 8. Since the K^+ influx rate at pH 7 in the presence of PheAsO plus mersalyl is substantially less than the K^+ efflux rate, there is a significant net loss of K^+ under these conditions. Due to the substantial rates of K^+ efflux, the unidirectional K^+ influx rates determined in the presence of

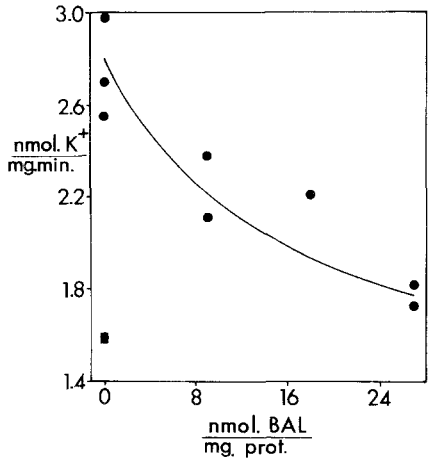

Fig. 2. Effect of BAL on the stimulation of K^+ influx by PheAsO. Unidirectional K^+ influx, determined from the change in labeled K^+ content between samples taken after 2 and 8 min of incubation, in units of nmol K^+ per mg protein per minute, is plotted against the concentration of BAL, added after 1 min of incubation, in units of nmol per mg protein. Symbols: ■, control samples; ●, the medium included from zero time 9.2 nmol. PheAsO per mg protein, or 50 μM.

PheAsO plus mersalyl must be considered approximate and are probably underestimates.

Figure 2 depicts the ability of BAL, added after the PheAsO, to block the stimulation of K^+ influx by PheAsO. Nearly total apparent reversal is obtained at a BAL concentration three times that of PheAsO. Effects of BAL and 2-ME on the stimulation of K^+ influx by PheAsO are compared in Table II. 2-ME was tested at twice the BAL concentration, in order to compare equivalent levels of sulfhydryl groups. 2-ME, added after the PheAsO, consistently causes some apparent reversal of the stimulation of K^+ influx by PheAsO.

Oxygen electrode measurements under essentially similar conditions have confirmed some earlier observations of uncoupling by arsenicals (Fluharty and Sanadi, 1962; Sanadi *et al.*, 1981). PheAsO, at levels comparable to those tested in the ^{42}K studies, stimulates respiration after a lag of about 5–7 min, the lag time being slightly shorter at higher PheAsO levels. Respiration rates from a representative experiment are shown in Table III. The stimulation of respiration is prevented by BAL, at twice the PheAsO concentration, added before (data not shown) or after the PheAsO. Consistent with earlier studies (Diwan *et al.*, 1983), BAL alone has no effect on respiration under the conditions studied. Reported inhibitory effects of BAL on respiratory chain activity require prolonged treatment with BAL at much

Table II. Effect of BAL and 2-ME on the Stimulation of K^+ Influx by PheAsO[a]

Other additions	K^+ influx (nmol K^+/mg protein per min)	
	− PheAsO	+ PheAsO
None	0.80	1.40
	1.03	1.66
	1.00	1.64
BAL	0.88	1.15
	1.06	1.11
2-ME	1.02	1.20
	1.00	1.23

[a]PheAsO when included was in the medium from zero time at 6.1 nmol per mg protein (50 μM). BAL, at 12 nmol per mg protein (100 μM), and 2-ME, at 24 nmol per mg protein (200 μM), when included were added after 1 min of incubation. Unidirectional K^+ influx rates were calculated from the change in ^{42}K content between samples taken after 2 and 8 min of incubation.

Table III. Effect of PheAsO, BAL, and 2-ME on Mitochondrial Respiration[a]

Additions (nmol/mg protein)			Respiration rate (ngatom O/mg protein per min)		
PheAsO	BAL	2-ME	Initial	5–6 min	2 min before anaerobiosis
—	—	—	15	16	14
—	—	—	15	17	15
8.5	—	—	15	16	27
8.5	—	—	15	19	21
17	—	—	15	21	36
17	—	—	15	21	32
17	34	—	16	17	14
17	34	—	15	15	15
17	—	68	16	29	33
17	—	68	15	28	33
—	34	—	16	16	14
—	34	—	16	19	16
—	—	68	15	15	14
—	—	68	17	17	16

[a]The medium contained 200 mM sucrose, 9.5 mM succinic acid, and 1.9 mM KOH, adjusted to pH 7.5 with Tris. The protein concentration was 2.2 mg/ml. When included, PheAsO was added 2 min after the mitochondria, and BAL or 2-ME was added 1 min after the PheAsO. The "initial" and "5–6 min" respiration rates depicted are the rates before PheAsO addition and 5–6 min after PheAsO addition.

Table IV. Effect of PheAsO and Mersalyl on K^+ Flux into Mitochondria Pretreated with DCCD[a]

	Additions	K^+ Influx (nmol/mg min)		Average % inhibition by DCCD
		Control mitochondria	DCCD-treated mitochondria	
A	None	1.17 ± 0.10 (3)	0.75 ± 0.04 (3)	36
	PheAsO	1.81 ± 0.05 (3)	1.15 ± 0.17 (3)	37
B	None	1.14 ± 0.15 (8)	0.72 ± 0.17 (8)	37
	Mersalyl	1.81 ± 0.11 (8)	1.14 ± 0.17 (8)	37

[a]In the experiment summarized in A, a portion of the mitochondrial stock suspension, which contained 41 mg protein/ml, was preincubated at 0°C for at least 45 min with 30 nmol DCCD per mg protein. PheAsO when present in the incubation medium was at 8.5 nmol per mg protein (45 μM). The data in B include combined values from two similar experiments. DCCD treatment was as in A. Mersalyl when included was in the medium at 150 μM (23 nmol/mg protein). The values shown are means of individual determinations ± standard deviations (number of determinations).

higher concentrations than those tested in the present studies (VanArk *et al.*, 1981; Zhu *et al.*, 1982). 2-ME does not block the effect of PheAsO, but rather shortens the lag time before the increase in respiration rate becomes evident. Other studies have shown that uncoupling by PheAsO is observed both in the presence and absence of K^+.

Table IV examines the effects of PheAsO and mersalyl on K^+ flux into mitochondria pretreated with DCCD. The results show that DCCD inhibits K^+ influx to the same extent either under control conditions or in the presence of PheAsO or mersalyl. An alternative way of assessing the data yields the comparison that PheAsO stimulates K^+ flux into control mitochondria an average of 55% in the experiment of Table IVA, compared to a 53% stimulation of K^+ flux into DCCD-treated mitochondria. The results in Table IVB similarly show an equivalent stimulation by mersalyl of K^+ flux into control mitochondria (58%) compared to the stimulation of K^+ flux into DCCD-treated mitochondria (59%).

Discussion

The results confirm the proposal (Sanadi *et al.*, 1981) that PheAsO activates K^+ entry into respiring mitochondria. Addition of either BAL or 2-ME, subsequent to PheAsO treatment, diminshes the stimulation of K^+ influx. Previous studies, carried out under somewhat different conditions, showed an earlier onset of PheAsO-induced K^+-dependent H^+ ejection in the presence of 2-ME (Sanadi *et al.*, 1981). The finding of substantial apparent reversal by the monothiol 2-ME of the stimulatory effect of PheAsO on K^+

influx suggests that the reactive group mediating this effect may be a monothiol rather than a dithiol. Usually reactions of organic arsenicals to form stable cyclic structures with dithiols are not readily reversed by treatment with monothiols (Johnstone, 1963; Knowles and Benson, 1983). Uncoupling effects of PheAsO and other arsenicals are not reversed by monothiols (Fluharty and Sanadi, 1963; data of Table III).

The differing sensitivity to 2-ME of effects on respiration and K^+ flux indicates that PheAsO affects K^+ influx by binding to some site distinct from that which mediates uncoupling by the arsenical. This conclusion is further supported by the finding that the uncoupling effects of PheAsO and other arsenicals exhibit a time lag (Fluharty and Sanadi, 1962; Sanadi *et al.*, 1981; present studies), whereas within the time resolution of the measurements no comparable delay is seen to precede the stimulation of K^+ influx by PheAsO. The decreasing rate of ^{42}K uptake and development of net K^+ loss at later times with the highest PheAsO concentration tested may reflect the onset of delayed uncoupling. Conventional uncouplers inhibit K^+ flux into respiring mitochondria, while stimulating K^+ efflux (Diwan and Tedeschi, 1975; Diwan and Lehrer, 1978). Separate sites of interaction of PheAsO with mechanisms mediating respiration-dependent K^+ influx and ATP synthesis would be consistent with the chemiosmotic theory, which predicts only indirect coupling between mitochondrial cation transport and energy transduction (Mitchell and Moyle, 1969; Jung *et al.*, 1977; Nicholls and Åkerman, 1982) According to the chemiosmotic model, the K^+-dependent external acidification observed by Sanadi and co-workers (Sanadi *et al.*, 1981) may be attributed to increased respiration-linked H^+ ejection as PheAsO activates K^+ entry, thereby diminishing the membrane potential.

The stimulatory effect of PheAsO on K^+ influx resembles the effect of the tin derivative DBCT in being sensitive to 2-ME (Diwan *et al.*, 1983). However, an inhibitory effect comparable to that caused by BAL in the presence of DBCT (Diwan *et al.*, 1983) is not observed when BAL is added in the presence of PheAsO.

No substantial additivity of effects of PheAsO and mersalyl on K^+ influx is seen; this is consistent with a common site of interaction of these reagents with the K^+ influx mechanism. The interaction of PheAsO and mersalyl with the K^+ efflux mechanism may be more complex, considering the apparent synergistic effect of these reagents in stimulating K^+ efflux. Differential effects on K^+ influx and efflux are consistent with the proposal (Jung *et al.*, 1977) that entry and exit of K^+ from mitochondria may involve separate mechanisms. Further support for this view is the finding that Ba^{2+}, an inhibitor of K^+ influx, fails to affect K^+ efflux (Diwan, 1985).

The results depicted in Table IV indicate that DCCD affects K^+ influx at some site other than the site(s) which react with PheAsO and mersalyl. The

K^+ influx mechanism of mitochondria pretreated with the covalently reactive DCCD exhibits unchanged sensitivity to PheAsO and to mersalyl. Thus the site(s) of interaction of DCCD with the K^+ influx mechanism must be separate from and independent of the sulfhydryl group(s) exhibiting sensitivity to PheAsO and mersalyl.

Acknowledgments

This work was supported by USPHS Grant GM-20726. Helpful discussions with Dr. D. R. Sanadi and the participation in some experiments of Ms. Pamela McGinnis are gratefully acknowledged.

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