Biochemical Pharmacology, Vol. 20, pp. 2417–2427. Pergamon Press, 1971. Printed in Great Britain

2-OXOACID DEHYDROGENASES OF RAT LIVER MITOCHONDRIA AS THE SITE OF ACTION OF S-(1,2 DICHLOROVINYL)-L-CYSTEINE AND S-(1,2 DICHLOROVINYL)-3-MERCAPTOPROPIONIC ACID

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(*Received* 21 *December* 1970; *accepted* 24 *March* 1971)

Abstract—DCVC and DCVMP, two structurally related compounds, inhibit respiration of rat liver mitochondria by interacting with the 2-oxoacid dehydrogenases. DCVC causes a progressive inhibition of respiration which is delayed in onset whilst DCVMP causes an immediate inhibition of respiration. In order to account for the differences in the form of inhibition it is suggested that DCVC and DCVMP may interact with different components of the 2-oxoacid dehydrogenases through different groupings.

A RELATIONSHIP between feeding soya bean meal which had been extracted with trichloroethylene and outbreaks of aplastic anaemia in cattle was postulated by Stockman[1] and the toxic agent tentatively identified as an amino-acid analogue: S-(1,2 dichlorovinyl)-L-cysteine [DCVC]. When synthesized and administered to calves, DCVC produced similar symptoms to those found by feeding soya bean meal extract.[2] DCVC shows species-specificity in its toxic action.[3,4] In the calf the major target organ is the haemopoietic system but there is also considerable kidney damage, whereas in the rat and several other laboratory species a renal tubular necrosis only was found.[5]

A biochemical study was undertaken by Parker[6] in which DCVC and a related compound, S-(1,2 dichlorovinyl)-3-mercaptopropionic acid [DCVMP] were shown to inhibit DNP-stimulated respiration of isolated rat liver and kidney mitochondria *in vitro*. When isolated 2–4 hr after i.p. administration of DCVC or DCVMP respectively, rat kidney mitochondria possessed impaired respiration. This inhibition of respiration by DCVC could not be reversed by washing the isolated mitochondria whereas addition of DCVC to normal isolated rat kidney mitochondria at 0° followed subsequently by washing caused apparent reversal of inhibition. This is probably related to metabolism of DCVC which will not occur at low temperature.

Due to the irreversible inhibition of respiration of rat kidney mitochondria caused by DCVC, further studies of the effects of these potential alkylating agents DCVC and DCVMP upon oxidative phosphorylation have been made. For reasons discussed later, these studies have been carried out with rat liver mitochondria rather than kidney mitochondria *in vitro*. This paper attempts to define the site(s) of action of DCVC and DCVMP in mitochondria.

In relation to this paper, the following paper will propose that DCVC has to be metabolized before exerting its inhibitory action.[7]

MATERIALS AND METHODS

Isolation of mitochondria

Rat liver mitochondria were prepared from whole liver in 300 mM sucrose as described by Aldridge.[8] A mitochondrial fraction from rat kidney was isolated similarly except that EDTA was added to the homogenization medium to give a final concentration of 1 mM.

Measurement of respiration

Oxygen uptake was measured manometrically according to Aldridge.[9] The basic reaction medium for mitochondria has already been described by Aldridge[8] and had the following composition: 100 mM KCl, 14 mM $MgCl_2$, 1 mM EDTA, 16·7mM potassium phosphate, pH 6·8 (3 ml total vol.). Reaction temperature 37°.

Measurement of ATP hydrolysis

ATP hydrolysis was measured using the medium and methods described by Aldridge and Stoner[10] with Fiske–Subbarrow reagent[11] to estimate inorganic phosphate (P_i) released.

Measurement of 2-oxoglutarate and citrate

2-oxoglutarate and citrate were measured in the supernatant obtained from mitochondrial suspensions precipitated with perchloric acid and neutralized with potassium carbonate. 2-oxoglutarate was assayed according to Bergmeyer[12] with glutamate dehydrogenase (EC 1.4.1.3) in 50% ammonium sulphate suspension. Citrate was assayed according to Moellering and Gruber[13] with citrate lyase (EC 4.1.3.6) and malate dehydrogenase (EC 1.1.1.37). All assays were performed spectrophotometrically at 25° using a Unicam SP 800.

Stabilisation of [1-^{14}C]pyruvic acid

[1-^{14}C]Sodium pyruvate (from the Radiochemical Centre, Amersham; 23·8 mc/m-mole), was stabilized by dissolution in a stoichiometric amount of hydrochloric acid to convert the salt to the free acid as described by Von Korff[14] and stored at 4°.

Measurement of [^{14}C]CO_2

[^{14}C]CO_2 was collected in the centre well of a Warburg flask and converted to barium carbonate according to procedure 1 of Cremer.[15] Filter papers were not present in the centre wells. The precipitate of barium carbonate was suspended in 9–12 ml of Cab-o-Sil/dioxan scintillator for counting. The scintillator had the following composition: toluene–1,4 dioxan–methyl cellosolve (1:3:3, by vol.); PPO (2,5 diphenyloxazole), 1% w/v; napthalene, 8% w/v; Cab-o-Sil; 4% w/v. Counting was carried out in Packard Tri-Carb 4000 Scintillation counter with a counting efficiency of 70 per cent determined from internal standards.

Protein estimation

Protein was estimated by the biuret reaction according to Robinson and Hogden[16] as modified by Aldridge.[17]

Special reagents

The following reagents were obtained from the sources indicated: Sigma Chemical Co; ATP (disodium salt from equine muscle), NADH (disodium salt), sodium pyruvate (TYPE II), 2-oxoglutaric acid, disodium succinate, sodium-DL-3-hydroxybutyrate, trisodium citrate, L-malic acid, apyrase (from potato, Grade I) and hexokinase (from yeast Type VI). Boehringer & Soehne: NAD^+ (Grade II), glutamate dehydrogenase, malate dehydrogenase, citrate lyase. British Drug Houses Ltd.; L-cystine, rotenone. 3-Mercaptopropionic acid from Koch–Light & Co. All other reagents were of Analar grade. DCVC and DCVMP were synthesized as described by Parker.[6]

Animals

All rats used in these studies were of the Porton strain and were fed *ad lib.* on MRC diet 41B.[18]

Statistical methods

(1) Significance testing of the mean results between controls and experimental mitochondria was made by Student's *t*-test.

(2) Where appropriate, comparison between results obtained from respiration experiments and those of ATP hydrolysis were made as follows: The mean results and their standard errors were expressed as percentages and the composite standard error calculated by use of the formula

$$\frac{N \pm n}{M \pm m} = \frac{N}{M} \pm \frac{N}{M}\left[\left(\frac{n}{N}\right)^2 + \left(\frac{m}{M}\right)^2\right]^{\frac{1}{2}},$$

where $N \pm n$ = mean and S.E. of experimental results and $M \pm m$ = mean and S.E. of control results.[19] The degree of significance to be attached to this comparison was made by the method of Snedecor[20] based upon the values being derived from a large sample.

RESULTS

Previous work by Parker[6] on measurement of respiration by rat kidney mitochondria isolated after DCVC or DCVMP administration to the intact animal has been repeated and extended (using doses of the compounds similar to Parker[6] and at 4 hr after dosing where the inhibition of respiration is marked). An uncoupling agent of oxidative phosphorylation, 2,4-dinitrophenol (DNP), has been used to study respiration separated from ATP synthesis. The addition of DNP not only stimulates respiration but also stimulates ATP hydrolysis (which is normally latent in intact mitochondria) in a reaction which is believed to represent a reversal of the terminal reactions involved in ATP synthesis.[21]

Table 1 shows that rat kidney mitochondria isolated 4 hr after administration of DCVC have an impaired response towards DNP-stimulated respiration (confirming previous results of Parker[6]), and DNP-stimulated ATP hydrolysis. In order to decide whether DCVC has its primary effect upon either respiration involving electron transport and the TCA cycle or ATP hydrolysis, it was found necessary to compare both sets of results upon a common basis. This has been achieved by recalculating the

TABLE 1. EFFECT OF DCVC, DCVMP ADMINISTRATION i.p. ON DNP-STIMULATED RESPIRATION AND ATP HYDROLYSIS OF RAT KIDNEY MITOCHONDRIA ISOLATED 4 hr AFTER DOSING

Treatment of animal	Respiration: ngatom O/min/mg protein Mean ± S.E.	(No. of obs.)	Mean ± S.E. (%)	ATP Hydrolysis: nmole P_i/min/mg protein Mean ± S.E.	(No. of obs.)	Mean ± S.E. (%)
Control	394 ± 8·9	(16)	100 ± 2·26	446 ± 13·3	(16)	100 ± 2·98
55 mg DCVC/kg	284* ± 4·5	(8)	72·1† ± 1·99	370* ± 3·3	(8)	82·9† ± 2·59
15 mg DCVMP/kg	228* ± 22·3	(8)	57·8 ± 5·75	410 ± 41·6	(8)	91·8 ± 9·73

* $P < 0.002$ with respect to controls.
† These two values have been compared as described in the text.

Respiration was measured in 3 ml basic reaction medium pH 6·8 containing 10 mM pyruvate, 1 mM fumarate, 2·3 mM ATP, 30 μM DNP, rat kidney mitochondria (2–3 mg protein) in 30 mM sucrose final. Readings were taken over 30 min at 37° after 10 min preincubation of medium and mitochondria.

ATP hydrolysis was measured in 3 ml basic reaction medium (omitting inorganic phosphate) containing 2·9 mM ATP, 16·7 mM glycylglycine, 30 μM DNP, rat kidney mitochondria (2–3 mg protein) in 30 mM sucrose final pH 6·8. Assay was performed over 10 min at 37° after 10 min pre-incubation of medium.

results as percentages and testing for the statistical significance of the differences between respiration and ATP hydrolysis experiment (Statistical Method 2). Using this method, it has been found that inhibition of DNP-stimulated respiration is significantly greater than that of ATP hydrolysis. In similar experiments after the administration of DCVMP, it is seen that DNP-stimulated respiration is inhibited by 42 per cent but that the inhibition of DNP-stimulated ATP hydrolysis (8 per cent) is insignificant.

Rat liver mitochondria isolated after administration of DCVC or DCVMP (same respective doses and time of isolation of mitochondria as used previously) exhibited similar effects to those of the kidney mitochondria (Table 2) except that in the case of

TABLE 2. EFFECT OF DCVC, DCVMP ADMINISTRATION i.p. ON DNP-STIMULATED RESPIRATION AND ATP HYDROLYSIS OF RAT LIVER MITOCHONDRIA ISOLATED 4 hr AFTER DOSING

Treatment of animal	Respiration: ngatom O/min/mg protein Mean ± S.E.	(No. of obs.)	Mean ± S.E. (%)	ATP Hydrolysis: nmole P_i/min/mg protein Mean ± S.E.	(No. of obs.)	Mean ± S.E. (%)
Control	159 ± 6·0	(16)	100 ± 3·74	252 ± 11·7	(16)	100 ± 4·64
55 mg DCVC/kg	125* ± 6·0	(8)	78·5‡ ± 4·75	222 ± 15·0	(8)	88·0‡ ± 7·23
15 mg DCVMP/kg	72† ± 3·0	(8)	44·8 ± 2·51	214 ± 20·0	(8)	84·8 ± 8·90

* $P < 0.01$ with respect to controls.
† $P < 0.002$ with respect to controls.
‡ These two values have been compared as described in the text.
Respiration and ATP hydrolysis were measured as described in the legend to Table 1. Rat liver mitochondria (3–4 mg protein) in 30 mM sucrose final were used in both measurements.

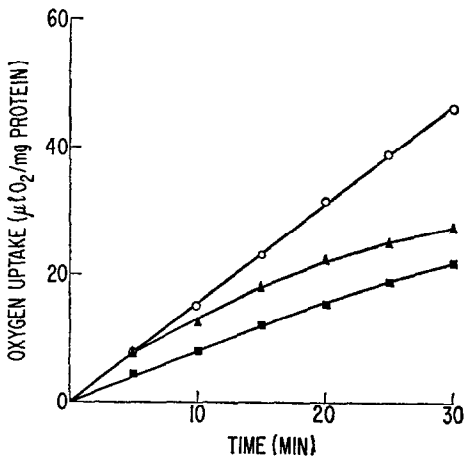

FIG. 1. Effect of DCVC, DCVMP upon DNP-stimulated respiration of rat liver mitochondria. Each flask contained 3 ml basic reaction medium pH 6·8. Additions made to this medium were 10 mM pyruvate, 1 mM fumarate, 2·3 mM ATP, 30 μM DNP, rat liver mitochondria (3–4 mg protein) in 30 mM sucrose final. Medium and mitochondria were preincubated for 10 min at 37° before the addition of DCVC or DCVMP.

○ Control, ▲ 100 μM DCVC, ■ 50 μM DCVMP.

DCVC the inhibition of DNP-stimulated respiration was not significantly lower than ATP hydrolysis when expressed on a percentage basis, although the inhibition of ATP hydrolysis was significantly lower than the control. This anomaly can probably be attributed to the large scatter in the values of both sets of results. These results suggest that *in vivo* both compounds primarily affect DNP-stimulated respiration rather than ATP hydrolysis and that in this respect liver mitochondria are as sensitive as kidney mitochondria.

Since respiration in both rat liver and kidney mitochondria is approximately equally inhibited after DCVC or DCVMP administration, it was decided to localize the site of action of DCVC and DCVMP in rat liver rather than rat kidney mitochondria *in vitro* since (i) they can be isolated more easily, intact, in better yield, and relatively uncontaminated (ii) they possess greater respiratory control (i.e. the initial low rate of respiration can be stimulated several fold by the addition of certain agents, e.g. apyrase, glucose-hexokinase phosphate acceptor system, uncouplers—see Aldridge).[8]

Comparison of phosphorylating and non-phosphorylating respiration *in vitro* using apyrase and DNP respectively (i.e. coupled and uncoupled systems) shows that respiration stimulated by DNP or by apyrase is inhibited by DCVC and DCVMP (Figs. 1 and 2); however, a key difference is apparent in the respective actions of DCVC and DCVMP upon respiration of rat liver mitochondria. Inhibition by DCVC of respiration in the presence of DNP is progressive but not immediate; there is a delay of at least 5 min before any inhibition can be detected (Fig. 1). The rate of progressive inhibition follows first-order kinetics (Fig. 3) when the substrate is pyruvate. The delay in the inhibition is greater when respiration is stimulated by the addition of apyrase (Fig. 2), approximately 15 min being required. In contrast, inhibition by DCVMP is immediate, followed by a progressive inhibition and the same form of curve is obtained whether respiration is stimulated by DNP or by apyrase (Figs. 1 and 2).

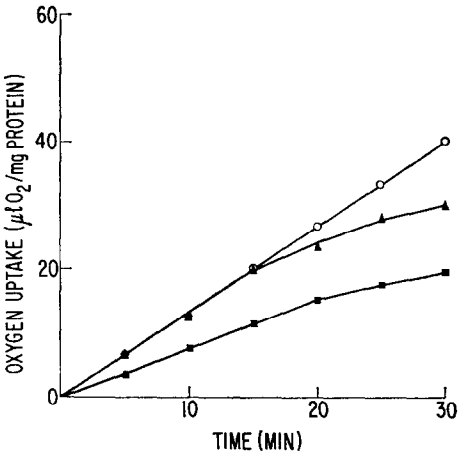

FIG. 2. Effect of DCVC, DCVMP upon apyrase-stimulated respiration of rat liver mitochondria. Each flask contained 3 ml basic reaction medium pH 6·8. Additions made to this medium were 10 mM pyruvate, 1 mM fumarate, 2·3 mM ATP, 0·65 unit purified apyrase, rat liver mitochondria (3–4 mg protein) in 30 mM sucrose final. Medium and mitochondria were preincubated for 10 min. at 37° before the addition of DCVC or DCVMP.
○ Control, ▲ 100 μM DCVC, ■ 50 μM DCVMP.

In order to define the sites of action of DCVC and DCVMP more closely a study has been made of the effects of these compounds upon the pathways of oxidation in rat liver mitochondria of the following substrates: pyruvate-fumarate, 2-oxoglutarate, succinate (+rotenone) and 3-hydroxybutyrate. The oxidation of succinate or 3-hydroxybutyrate stimulated by DNP, after a slight immediate inhibition, is relatively

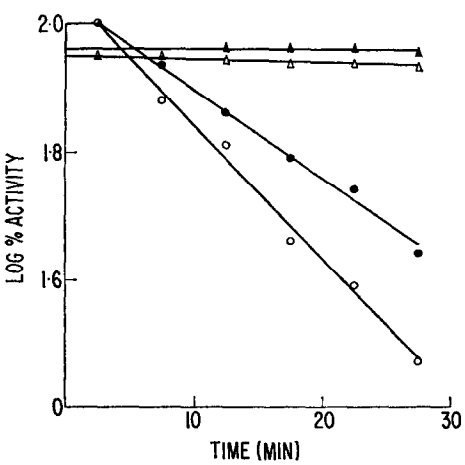

FIG. 3. Semilog plot of the effect of 100 μM DCVC upon oxidation of various substrates by rat liver mitochondria. Each flask contained 3 ml basic reaction medium, pH 6·8. Additions made to this medium were 10 mM substrate (20 mM for DL-3-hydroxybutyrate), 2·3 mM ATP, 30 μM DNP (only 10 μM DNP with succinate as substrate), rat liver mitochondria (3–4 mg protein) in 30 mM sucrose final. Medium and mitochondria were preincubated for 10 min at 37° before the addition of DCVC.
▲ Succinate (+1·1 μM rotenone), △ 3-Hydroxybutyrate, ● 2-Oxoglutarate, ○ Pyruvate-fumarate (1 mM).

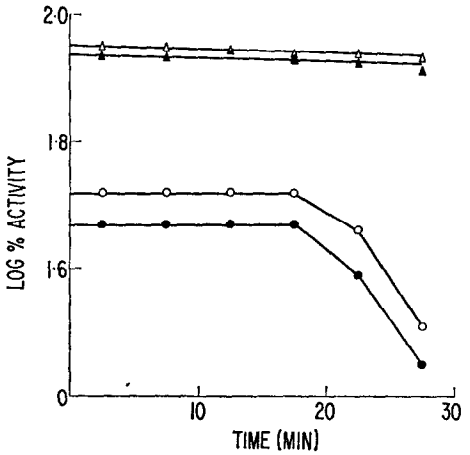

FIG. 4. Semilog plot of the effect of 50 μM DCVMP upon oxidation of various substrates by rat liver mitochondria. Each flask contained 3 ml basic reaction medium, pH 6·8. Additions made to this medium were 10 mM substrate (20 mM for DL-3-hydroxybutyrate), 2·3 mM ATP, 30 μM DNP (only 10 μM DNP with succinate as substrate), rat liver mitochondria (3–4 mg protein) in 30 mM sucrose final. Medium and mitochondria were preincubated for 10 min at 37° before the addition of DCVMP.

▲ Succinate (+1·1 μM rotenone), △ 3-Hydroxybutyrate, ● 2-Oxoglutarate, ○ Pyruvate-fumarate (1 mM).

unaffected. In contrast, with pyruvate or 2-oxoglutarate as substrate, DCVC after a short delay progressively inhibits DNP-stimulated respiration (Fig. 3) and DCVMP produces a strong immediate inhibition followed later by a progressive inhibition (Fig. 4). DCVC and DCVMP appear therefore not to act at a site in the electron transport chain common to all NAD^+ and flavoprotein linked dehydrogenases.

The oxidation of pyruvate–fumarate by rat liver mitochondria *in vitro* stimulated with apyrase leads to a linear accumulation of citrate over 30 min; this accumulation is prevented by the addition of DCVC or DCVMP (Table 3). When rat liver mitochondria oxidise citrate–L-malate *in vitro*, 2-oxoglutarate accumulates during the initial 10 min and the level only rises slowly in the next 20 min. Addition of DCVC or DCVMP to this system causes a continual increase in the level of 2-oxoglutarate over 30 min (Table 3). These observations are consistent with inhibition of 2-oxoacid dehydrogenases by DCVC or DCVMP.

Confirmation of the above view has been obtained by measuring $[^{14}C]CO_2$ production from rat liver mitochondria oxidising [1-^{14}C]pyruvic acid in the presence of DCVC or DCVMP using the glucose-hexokinase phosphate acceptor system to stimulate respiration (Table 4). Although the production of $[^{14}C]CO_2$ is linear over 30 min in the control there is a diminished production of $[^{14}C]CO_2$ in the presence of DCVC or DCVMP. Since $[^{14}C]CO_2$ is split out from [1-^{14}C]pyruvic acid by the pyruvate dehydrogenase multi-enzyme complex, the action of DCVC and DCVMP must be upon this enzyme complex.

The *in vivo* administration of DCVC or DCVMP produces a slight inhibition of DNP-stimulated ATP hydrolysis by rat liver mitochondria and therefore observations have been made of the *in vitro* effect of these compounds upon ATP hydrolysis by rat liver mitochondria. The delayed effect of DCVC upon mitochondrial respiration

TABLE 3. EFFECT OF DCVC, DCVMP ON (i) CITRATE FORMATION BY RAT LIVER MITOCHONDRIA OXIDISING PYRUVATE–FUMARATE AND (ii) 2-OXOGLUTARATE FORMATION BY RAT LIVER MITOCHONDRIA OXIDISING CITRATE–L-MALATE

Time (min)	Citrate (nmole/mg protein) production			2-Oxoglutarate (nmole/mg protein) production		
	Control	100 μM DCVC	70 μM DCVMP	Control	100 μM DCVC	70 μM DCVMP
10	185 ± 19 (5)	117 ± 13 (5)	28* ± 7 (5)	29 ± 1 (6)	50* ± 2 (5)	47* ± 2 (6)
20	400 ± 32 (5)	161* ± 9 (5)	55* ± 5 (4)	35 ± 3 (6)	100* ± 5 (6)	73* ± 2 (5)
30	566 ± 50 (5)	133* ± 9 (5)	61* ± 5 (5)	40 ± 3 (6)	136* ± 9 (6)	99* ± 8 (6)

Results expressed as mean ± S.E. with number of observations in parentheses.
* $P < 0.001$.

Each flask contained 3 ml basic reaction medium pH 6·8. Additions made to this medium were 10 mM/1 mM substrate pair, 2·3 mM ATP, 0·65 unit purified apyrase (SIGMA), rat liver mitochondria (3–4 mg protein) in 30 mM sucrose final and DCVC (100 μM) or DCVMP (70 μM). Flasks were incubated at 37° on a metabolic shaker for specified times and reaction stopped by the addition of perchloric acid. Mitochondria were added after 10 min pre-incubation of medium.

suggested that the compound requires to be metabolised before inhibition can take place. Therefore, the experiments upon ATP hydrolysis have been conducted after various times of preincubation of the mitochondria with DCVC before the addition of DNP.

Table 5 shows that ATP hydrolysis by rat liver mitochondria is stimulated by DCVMP linearly but by DCVC only after a delay. When ATP hydrolysis in the absence of DNP is subtracted from total ATP hydrolysis, it is seen that DNP-stimulated ATP hydrolysis is unaffected by DCVC but is reduced by approximately 35 per cent by DCVMP (Table 5).

TABLE 4. EFFECT OF DCVC, DCVMP ON [^{14}C]CO_2 OUTPUT FROM RAT LIVER MITOCHONDRIA OXIDIZING [1-^{14}C]PYRUVIC ACID

Time (min)	dis./min [^{14}C]CO_2/mg protein		
	Control	100 μM DCVC	70 μM DCVMP
10	2165 ± 97 (4)	1343* ± 179 (3)	932† ± 98 (2)
20	4062 ± 237 (4)	2077* ± 394 (3)	1150† ± 140 (2)
30	5959 ± 262 (8)	2533† ± 578 (3)	1277† ± 105 (4)

Results expressed as mean ± S.E. Number of observations in parentheses.
* $P < 0.01$.
† $P < 0.001$.

Each flask contained 3 ml basic reaction medium pH 6·8. Additions made to this medium were 10 mM pyruvate, 1 mM fumarate, 2·3 mM ATP, 60 mM glucose, 40 units hexokinase (SIGMA), 16·7 mM glycylglycine, rat liver mitochondria (3–4 mg protein) in 30 mM sucrose final. Ten min pre-incubation of mitochondria at 37° with DCVC (100 μM) or DCVMP (70 μM) was followed by the addition of [1-^{14}C]pyruvic acid (approx. 2×10^5 dis./min) to each flask. [^{14}C]CO_2 was collected in the centre well of each Warburg flask.

TABLE 5. EFFECT OF 100 μM DCVC, 50 μM DCVMP ON UNSTIMULATED AND DNP-STIMULATED ATP HYDROLYSIS OF ISOLATED RAT LIVER MITOCHONDRIA

Time (min)	A μmole P_i/mg protein: Total ATP hydrolysis (unstim. + 30 μM DNP)			B μmole P_i/mg protein: ATP hydrolysis (unstimulated)			C μmole P_i/2 min/mg protein: ATP hydrolysis (30 μM DNP only)		
	Cont.	DCVC	DCVMP	Cont.	DCVC	DCVMP	Cont.	DCVC	DCVMP
12	0·89 ± 0·036	0·88 ± 0·035	1·04 ± 0·047	0·019 (0·023, 0·014)	0·025 (0·021, 0·029)	0·453 (0·304, 0·456, 0·590)	0·87	0·85	0·59
22	0·94 ± 0·046	0·99 ± 0·051	1·59 ± 0·060	0·058 (0·054, 0·061)	0·160 (0·141, 0·179)	1·08 (1·14, 1·02)	0·88	0·83	0·51
32	0·96 ± 0·068	1·27 ± 0·12	2·13 ± 0·070	0·126 (0·148, 0·104)	0·429 (0·450, 0·408)	1·59 (1·54, 1·64)	0·83	0·84	0·54
	Mean ± S.E. (4 obs. at each time)			Mean of two observations at each time					

ATP hydrolysis was measured as described in legend to Table 1 with the following modifications. Unstimulated ATP hydrolysis was measured over the times stated but ATP hydrolysis in the presence of 30 μM DNP was measured only over 2 min in the same assay system by adding DNP during the last 2 min of each assay time. ATP hydrolysis in the presence of 30 μM DNP (C) was calculated by subtracting unstimulated (B) mean values from total mean values (A).

DISCUSSION

Both DCVC and DCVMP inhibit DNP-stimulated respiration of rat liver and kidney mitochondria isolated 4 hr after their administration to the intact animal. Whilst DCVMP administration strongly inhibits respiration of rat liver and kidney mitochondria, only slight inhibition is seen upon ATP hydrolysis. With DCVC the difference between respiration and ATP hydrolysis is not so clear. Firstly, in rat kidney mitochondria, both respiration and ATP hydrolysis stimulated by 30 μM DNP are significantly inhibited. Secondly, in rat liver mitochondria, inhibition of respiration by DCVC is significantly inhibited whilst ATP hydrolysis is not.

The sites of action of DCVC and DCVMP have been elucidated in a systematic study upon rat liver mitochondria *in vitro*. Measurements of oxygen uptake, carbon dioxide output and selected TCA cycle intermediates (Tables 3 and 4 and Figs. 3 and 4) demonstrate that the site of action of DCVC and DCVMP is at the 2-oxoacid dehydrogenases. From these measurements, it is apparent that the time courses of action of DCVC and DCVMP are different; DCVMP appears to act immediately whilst the action of DCVC is less immediate. Both compounds are shown to affect ATP hydrolysis in rat liver mitochondria *in vitro*; DCVC causes a slight stimulation of ATP hydrolysis which is delayed in onset and is therefore similar to its delayed action upon respiration, and further supports the concept that the metabolism of DCVC to an active inhibitor may be required, but DCVC does not cause inhibition of ATP hydrolysis stimulated by DNP. The immediate action of DCVMP is similar to that observed upon respiration.

Pyruvate and 2-oxoglutarate dehydrogenase complexes have several features in common, one of which is that they share the same cofactor requirements and of the three enzymes in each complex, only one is common to both. These complexes are associated with many -SH containing compounds, e.g. CoA, lipoic acid and a flavoprotein, lipoyl dehydrogenase ($NADH_2$: lipoic acid oxidoreductase EC 1.6.4.3), with an active centre postulated to contain FAD and a redox-disulphide grouping.[22] This enzyme is common to both complexes. Such thiols may be potential acceptors for DCVC and DCVMP although Parker[6] could not find any evidence to support this suggestion.

A preliminary observation has suggested that lipoyl dehydrogenase may be the biological acceptor for DCVC or a metabolite. Only under conditions in which DCVC can be metabolised by rat liver mitochondria, can inhibition of lipoyl dehydrogenase activity be demonstrated; no inhibition is seen with DCVMP (Stonard, unpublished observations).

The differences in the time course and form of inhibition by DCVC and DCVMP of respiration of rat liver mitochondria and the observation that DCVMP is without effect upon lipoyl dehydrogenase activity prompts the suggestion that the sites of action of DCVC and DCVMP within 2-oxoacid dehydrogenases are different. The most significant difference is the delayed onset of inhibition of respiration seen with DCVC. The following paper is devoted to the metabolism of DCVC and its relevance to the inhibition of respiration of rat liver mitochondria *in vitro*.[7]

The distinct differences in the form of inhibition of respiration by DCVC and DCVMP respectively may be related to the metabolism of DCVC. However, different functional groups may be active in the respective molecules. In the latter respect, preliminary evidence suggests that DCVMP may interact with CoA since DCVMP appears to be treated as a fatty acid by rat liver mitochondria; (Stonard, unpublished

observations). CoA is an essential component of the 2-oxoacid dehydrogenases and the initial step of fatty acid oxidation is activation to the CoA ester. Therefore, a possible mode of action of DCVMP emerges.

Further experiments are therefore being carried out to confirm whether or not, DCVC and DCVMP, two structurally related compounds interact with different components of the 2-oxoacid dehydrogenase complexes through different groupings.

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