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UNCOUPLING OF OXIDATIVE PHOSPHORYLATION IN RAT LIVER MITOCHONDRIA FOLLOWING THE ADMINISTRATION OF DIMETHYL SULPHOXIDE

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A single intraperitoneal injection of 275 mg of dimethyl sulphoxide (DMSO) to rats (100-125 g body weight) effectively uncouples oxidative phosphorylation in liver mitochondria during the period from 2 hr to 5 day post-injection. Higher doses of DMSO are inhibitory to mitochondrial respiration. DMSO has however no uncoupling action on oxidative phosphorylation in vitro. On the other hand, dimethyl sulphide (DMS), a known metabolite of DMSO, brings about the uncoupling effect in vitro. The uncoupling of oxidative phosphorylation by normal mitochondria could also be achieved if these are pre-incubated (30 min at 0^{o}C) with the post-mitochondrial liver supernatant derived from rat injected with DMSO, 2-24 hr prior to sacrifice. These results provide explanation for the observed uncoupling effect exerted by DMSO in vivo.

Dimethyl sulphoxide (DMSO) is used as a solvent for a wide range of chemicals including those subjected to pharmacological and toxicological investigations. However, DMSO by itself is shown to possess several pharmacological properties (1). In toxicology studies, DMSO is found to be less toxic as compared to other chemicals since large doses of DMSO are required to cause noticeable toxicity in animals (2). DMSO is also shown to possess radioprotective and cryoprotective properties (3). The chemical has been used as a vehicle for other medicinal agents and drugs to penetrate through the skin (4-6). Although several studies have been carried out on the therapeutic value of DMSO and also about its other applications, practically nothing is known about the changes that may be brought about at the level of sub-cellular organelles upon in vivo administration of this chemical. The present studies pertain to alterations brought about by DMSO in liver mitochondrial function. It was observed that a single i.p. injection

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of 0.25 ml (275 mg/100-125 g body weight) of DMSO to rats could effectively uncouple oxidative phosphorylation in liver mitochondria. The effect is perceptible as early as 2 hr following the intraperitoneal injection and remains upto 5 days. DMSO does not uncouple oxidative phosphorylation in vitro, but the addition of dimethyl sulphide (DMS), a known metabolite of DMSO, could result in uncoupling of oxidative phosphorylation.

MATERIALS AND METHODS

Chemicals: DMSO was obtained from Cambriam Chemicals, U.K., DMS was purchased from E.Merck, W. Germany, ADP was purchased from Sigma Chemical Company, U.S.A. All other chemicals used in the studies were of Analar grade.

Treatment of the animals: Male albino rats of Wistar strain, each weighing 100 to 125 g and fed on laboratory stock diet, were used. The animals were administered intraperitoneally varying quantities of DMSO and sacrificed at different time intervals. Diet and water were made available ad libitum to both control and DMSO injected rats.

Isolation of mitochondria: Mitochondria were isolated essentially as described by Katyare et al. (7).

Determination of oxidative phosphorylation: Measurements on oxygen consumption and oxidative phosphorylation were carried out at 25°C in a total volume of 1.3 ml in a Gilson oxygraph, model KM (Gilson Medical Electronics, U.S.A.) using a Clark-type oxygen electrode. The reaction mixtures employed for oxygen consumption was essentially that described by Potter (8) and that for oxidative phosphorylation as described by Satav and Katyare (9). In oxidative phosphorylation studies small aliquots of ADP (approximately 100-200 n moles in 15 µl) were added and rates of respiration in the presence of ADP (State 3) and after its depletion (State 4) were recorded. ADP/O ratio and respiratory control index (RCI) values were calculated as described by Satav and Katyare (9).

Protein determination was carried out according to Lowry et al. (10) using crystalline bovine serum albumin as a standard.

RESULTS AND DISCUSSION

In the initial studies aspects relating to the effect of DMSO on the rates of oxygen consumption were determined. These results are depicted in Fig.1. It can be seen that maximum rates of oxygen uptake by liver mitochondria are seen in the case of rats administered the dose of 0.25 ml of DMSO (275 mg) at 2 hr prior to sacrifice. At higher doses of DMSO, oxygen uptake by liver mitochondria is inhibited. The

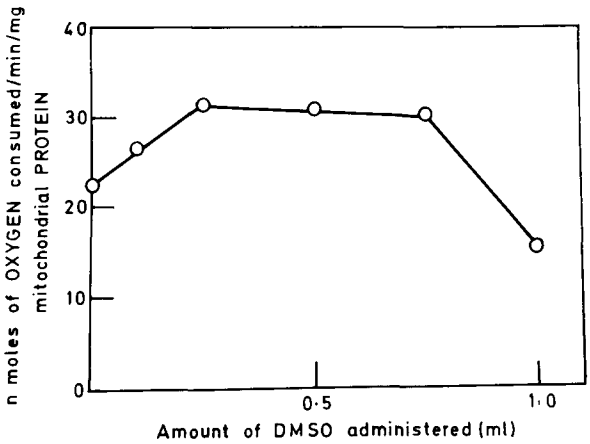

Figure 1. Dose dependent effects of DMSO on oxygen consumption by rat liver mitochondria. Rats weighing 100-125 g each were administered intraperitoneally with various amounts of DMSO and sacrificed after 2 hr for isolation of liver mitochondria. Other details as in materials and methods.

dose of 275 mg of DMSO is very much less than the toxic dose (15 g/kg body weight) reported for rats (2,11). Results of the time-course experiment with this dose of DMSO are shown in Fig.2. It can be seen that the DMSO elicits a significant rise in the rates of oxygen consumption by liver mitochondria as early as at 2 hr post-injection period and the oxygen uptake levels remain elevated as long as 5 days post-injection before reverting to the normal level by the 7th day.

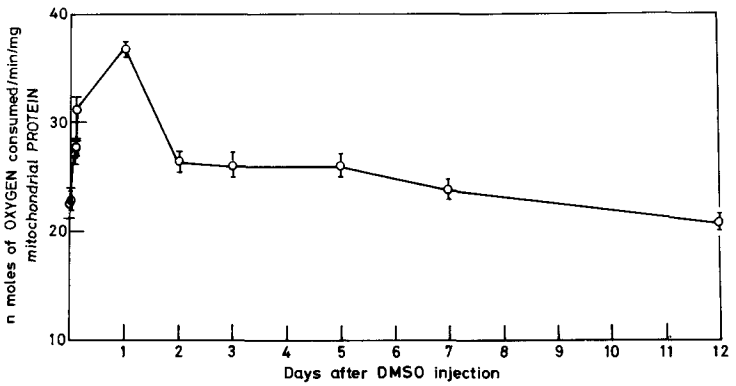

Figure 2. Effect of DMSO on the oxygen consumption by rat liver mitochondria at various times post-injection. Rats weighing 100-125 g each were administered intraperitoneally with 0.25 ml of DMSO (275 mg) and sacrificed at the times indicated in the figure. Three-four rats were used at each time point. Vertical bars represent ± SEM.

Table I

ADP/O ratio and RCI (using succinate as substrate) by rat liver mitochondria at various periods following DMSO injection

Post-injection period	ADP/O ratio	Rate of oxidation (n moles O_2/min/mg protein) +ADP	-ADP	RCI
Control	1.62 ± 0.06	49.0 ± 4.27	23.5 ± 2.91	2.085
2 hr	0.56 ± 0.03	55.3 ± 3.24	32.0 ± 1.43	1.728
24 hr	0.75 ± 0.12	84.11 ± 7	52.66 ± 5.6	1.60
48 hr	0.76 ± 0.15	56.5 ± 3.2	30.0 ± 2.75	1.88
3 day	0.53 ± 0.09	55.36 ± 2.15	35.0 ± 2.23	1.58
5 day	0.57 ± 0.02	46.9 ± 1.12	30.45 ± 1.83	1.54
7 day	1.66 ± 0.11	66.05 ± 5.58	34.83 ± 1.94	1.90
12 day	1.77 ± 0.08	45.37 ± 1.90	23.55 ± 1.01	1.93

Rats weighing 100-125 g were administered with 0.25 ml of DMSO (275 mg) intraperitoneally and sacrificed at various intervals. Each group consisted of 3-4 rats. The results are expressed as ± S.E.M.

Results given in Table I show that the *in vivo* administration of DMSO to rats effectively uncouples oxidative phosphorylation, evidenced by a decrease in the ADP/O ratio and fall in the respiratory control index (RCI). As observed in the oxygen uptake studies, the uncoupling of oxidative phosphorylation is also seen during the period from 2 hr to 5 days following DMSO injection. Thereafter, the liver mitochondrial oxidative phosphorylation is returned to the normal value.

Studies on the fate of DMSO *in vivo* indicate that metabolites of DMSO are retained by cellular proteins in tissues for longer periods than DMSO (12) and hence most of the alterations elicited by DMSO *in vivo* can be ascribed to the metabolites of DMSO. We studied the *in vitro* effects of DMSO, its principal metabolite DMS and the liver post-mitochondrial supernatant obtained from rats administered DMSO 2 and 24 hr before sacrifice, on the liver mitochondrial functions. It can be seen from the data given in Table II that DMSO by itself has no effect on mitochondrial oxidative phosphorylation. However, DMS effectively increases the rate of oxygen consumption and uncouples the

Table II

Effect of DMSO and its metabolites in vitro on ADP/O ratio and RCI by isolated rat liver mitochondria

Additions/ pretreatments	ADP/O ratio	Rate of oxygen uptake (n moles of O_2/min/mg protein) +ADP	-ADP	RCI
None	1.62 ± 0.06	49.00 ± 4.27	23.50 ± 2.91	2.09
DMSO	1.47 ± 0.09	70.66 ± 2.90	30.66 ± 1.90	2.30
DMS	0.47 ± 0.06	77.84 ± 5.02	42.85 ± 4.12	1.82
Mitochondria pre-incubated with post-mitochondrial supernatant obtained from 2 hr post-injected rats	1.12 ± 0.05	40.00 ± 2.60	23.95 ± 2.77	1.67
Mitochondria pre-incubated with post-mitochondrial supernatant obtained from 24 hr post-injected rats	0.86 ± 0.08	56.15 ± 2.57	31.34 ± 2.44	1.79

For in vitro studies 10 µl of DMSO or its metabolite DMS was included in the assay mixture and ADP/O ratio and RCI were calculated. In other sets of experiments mitochondria derived from control rat livers were incubated for 30 min at 0°C with post-mitochondrial liver supernatant from rats administered with 0.25 ml (275 mg) of DMSO, at 2 and 24 hr post-injection periods. The results are expressed as ± S.E.M.

oxidative phosphorylation as can be inferred from the decrease in ADP/O ratio and loss of RCI. Incubation of mitochondria from normal rat liver with the post-mitochondrial supernatants from DMSO-administered rats also elevates the rate of oxygen consumption and uncouples oxidative phosphorylation. Studies on retention time of the two metabolites of DMSO, DMS and $DMSO_2$ (dimethyl sulfone), have revealed that the metabolites remain in the body for about 2 weeks following administration of DMSO (13). The non-metabolized DMSO is excreted out in the urine much earlier than are its metabolites (13).

It would be of interest to probe into the mechanism of uncoupling of oxidative phosphorylation by DMS in vitro. Several explanations have been offered for the mode of action of the uncouplers. Racker has suggested that uncouplers act by causing dissociation of the coupling factor from respiratory chain (14). According to Pinchot (15),

dinitrophenol acts by inhibiting reassociation of the coupling enzyme with the electron transport chain rather than by hydrolysis of high-energy intermediates of oxidative phosphorylation. It has been suggested that uncouplers bring about conformational changes in the mitochondrial proteins (16,17). According to the chemiosmotic theory (18,19) proposed by Mitchell uncouplers act by discharging transmembrane proton gradient. DMSO is known to act on membrane-associated processes in vivo. This action may probably be exerted through its metabolites. It is hence possible that DMS may cause structural alterations in the mitochondrial membranes and thereby bring about the uncoupling effect.

DMSO has been used as a solvent for testing a variety of mutagens and carcinogens in animals. It seems that researchers are not aware of subtle changes that DMSO may itself exert at the mitochondrial level. In this context it is pertinent to mention recent reports (20-22) suggesting that quite a few mutagens and carcinogens may elicit alterations in mitochondrial functions(particularly at the level of mitochondrial DNA) before causing alterations in nuclear DNA. DMSO may facilitate such action of chemical mutagens at mitochondrial level. In fact, present studies were prompted by an unexpected observation that the oxygen uptake by liver mitochondria was increased in the DMSO blanks employed for studies on the effects of chemical mutagens (dissolved in DMSO) on mitochondrial functions.

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