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MODE OF ACTION OF GARLIC OIL—EFFECT ON OXIDATIVE PHOSPHORYLATION IN HEPATIC MITOCHONDRIA OF MICE

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Abstract—The effect of garlic oil on oxidative phosphorylation in liver mitochondria of mice has been studied. Garlic oil impairs oxidative phosphorylation in hepatic mitochondria. The coupling activity of sites 1, 2 and 3 appears to be affected by garlic oil. The extent to which respiration is affected varies with the substrates used. For example, garlic oil affects respiration during oxidation of NAD-linked substrates such as glutamate to a greater extent than during oxidation of succinate and ascorbate. The inhibitory effect on respiration is less than that on phosphorylation. Diallyl disulfide, the principal insecticidal component of garlic oil, has a similar inhibitory effect on the mitochondrial oxidative phosphorylation. In contrast, dipropyl disulfide, which is chemically comparable to diallyl disulfide but is devoid of insecticidal activity, has a substantially lesser effect on oxidative phosphorylation.

GARLIC OIL has been shown to be highly effective against a variety of insect pests.[1] The insecticidal property of garlic oil has been attributed to its two components, viz. diallyl disulfide and diallyl trisulfide.[2] A wide range of medicinal as well as antihelminthic, antiprotozoal and antimicrobial properties has also been ascribed to garlic (*Allium sativum* Linn.).[3–6] A recent study has shown that both garlic oil and diallyl disulfide inhibit protein synthesis in mosquito larvae.[7] Contrary to the effect on protein synthesis, the oxygen uptake by mosquito larvae is only marginally affected by garlic oil.[7] This prompted us to probe into the effect of garlic oil on oxidative metabolism with a view to elucidating the mode of action of garlic oil. This study is on the effect of garlic oil, diallyl disulfide and dipropyl disulfide on oxidative phosphorylation in hepatic mitochondria of mice. We chose hepatic mitochondria because the system is well investigated and understood.

MATERIALS AND METHODS

Chemicals. ATP, cytochrome c, hexokinase, L-glutamic acid, sodium ascorbate and sodium succinate were obtained from Sigma Chemical Co. The other chemicals including EDTA, glucose and sucrose were of "AnalaR" grade, products of British Drug Houses. Garlic oil, diallyl disulfide and dipropyl disulfide were prepared in the laboratory. These last-named compounds were emulsified with a small drop of Tween 80 and diluted with 0·9% NaCl before use.

Animals. Male Swiss albino mice weighing approximately 20 g (45 ± 2-days-old) were used. The animals were maintained on a nutritionally sufficient stock diet and water, given *ad lib.* The mice were sacrified by cervical dislocation. The liver was excized quickly and transferred into ice-cold 0·25 M sucrose solution for subsequent preparation of mitochondria.

Isolation of mitochondria. Mitochondria were isolated by differential centrifugation following the procedure of Dingle.[8] The mitochondria were washed in 0·25 M sucrose and resuspended in either 0·25 or 0·075 M sucrose following their isolation. Intact mitochondria, at a concentration corresponding to 24 mg protein/ml. were used in all experiments.

Determination of oxidative phosphorylation. Oxidative phosphorylation associated with the oxidation of glutamate and ascorbate was determined according to the method of Lehninger *et al.*[9] with minor modifications. The P/O ratio during the oxidation of succinate was measured using the method of Yost *et al.*[10] (Details of the composition of the reaction media are given in the legend to Tables 1–3.) The experimental flasks contained, in addition to the standard components in the main compartment, different concentrations of garlic oil, diallyl disulfide and dipropyl disulfide in the side arm. An equal volume of Tween 80 and physiological saline was added in the side arm of the control flasks. Oxygen consumption and phosphorus esterifications were studied in a Warburg respirometer (Gilson differential respirometer, Gilson Medical Electronics, Middleton, Wis., U.S.A.) during a 30-min incubation period at 25°. The flasks were allowed to equilibrate for 10 min and readings were taken for 30 min after tipping in the contents of the side arm. After the incubation period, the flasks were removed quickly and chilled by placing in cracked ice. The reaction was stopped by the addition of 0·5 ml of 10% trichloroacetic acid. Inorganic phosphate (Pi) was assayed employing the method of Lowry and Lopez.[11] The mitochondrial protein was determined by the method of Lowry *et al.*[12]

RESULTS

The effect of garlic oil, as a function of concentration, on oxidative phosphorylation during oxidation of glutamate, succinate and ascorbate is summarized in Tables 1, 2 and 3 respectively. During the oxidation of glutamate, respiration was inhibited by 55 and 63 per cent in the presence of 0·5 and 1·0 μl garlic oil respectively (Table 1). At these concentrations, the P/O ratio was decreased by 52 and 71 per cent

TABLE 1. EFFECT OF GARLIC OIL ON OXIDATIVE PHOSPHORYLATION IN HEPATIC MITOCHONDRIA OF MICE USING GLUTAMATE AS SUBSTRATE*

Concn of garlic oil (μl)	O_2 uptake (μatoms/min per mg protein × 10^2)	Pi esterified (μatoms/min per mg protein × 10^2)	P/O ratio	Per cent of control P/O ratio
0 (control)	1·66 ± 0·11	5·06 ± 0·38	3·04 ± 0·08	100
0·1	1·50 ± 0·15	4·11 ± 0·36	2·76 ± 0·05	91
0·5	0·75 ± 0·04	1·09 ± 0·07	1·45 ± 0·05	48
1·0	0·62 ± 0·04	0·54 ± 0·06	0·87 ± 0·07	29

* Each flask contained phosphate buffer (75 μmoles; pH 7·4), $MgCl_2$ (15 μmoles), EDTA (3 μmoles), ATP (7·5 μmoles), KF (39 μmoles), cytochrome c (0·03 μmole) and 0·5 ml of mitochondrial suspension corresponding to 12 mg protein in 0·25 M sucrose in the main compartment and hexokinase (5 mg), glucose (75 μmoles) and glutamate (30 μmoles) in the side arm, all in a total fluid volume of 3 ml. The center well contained 0·2 ml of 20% KOH with a small roll of Whatman No. 1 filter paper. Values are means ± S.E. based on four independent determinations. Experimental conditions were as described in Materials and Methods.

respectively. Respiration and phosphorylation were not affected to any great extent at a level of 0·1 μl (Table 1).

With succinate as substrate, the reduction in respiration amounted to only 18 per cent in the presence of 1·0 μl garlic oil (Table 2). Phosphorylation, however, was suppressed to a greater extent, resulting in 68 per cent inhibition at the level of 1·0 μl garlic oil (Table 2).

TABLE 2. EFFECT OF GARLIC OIL ON OXIDATIVE PHOSPHORYLATION IN HEPATIC MITOCHONDRIA OF MICE USING SUCCINATE AS SUBSTRATE*

Concn of garlic oil (μl)	O_2 uptake (μatoms/min per mg protein)	Pi esterified ($\times 10^{-2}$)	P/O ratio	Per cent of control P/O ratio
0 (Control)	1·73 ± 0·11	3·22 ± 0·25	1·86 ± 0·05	100
0·1	1·71 ± 0·10	2·99 ± 0·17	1·75 ± 0·06	94
0·5	1·73 ± 0·15	1·72 ± 0·15	1·00 ± 0·11	54
1·0	1·42 ± 0·16	0·70 ± 0·16	0·59 ± 0·07	32

* Each flask contained phosphate buffer (36 μmoles; pH 7·4), $MgSO_4$ (15 μmoles), ATP (6 μmoles), KF (65 μmoles), cytochrome c (0·09 μmole), sucrose (250 μmoles) and 0·5 ml of mitochondrial suspension corresponding to 12 mg protein in 0·25 M sucrose in the main compartment and hexokinase (5 mg), glucose (75 μmoles) and succinate (30 μmoles) in the side arm, all in a total fluid volume of 3 ml. The center well contained 0·2 ml of 20% KOH with a small roll of Whatman No. 1 filter paper. Values are means ± S.E. based on four independent determinations. Experimental conditions were as described in Materials and Methods.

During the oxidation of ascorbate, respiration was not affected by garlic oil at the concentration used, whereas phosphorylation was inhibited (Table 3). The reduction in phosphorylation amounted to 18, 64 and 79 per cent at 0·1, 0·5 and 1·0 μl of garlic oil respectively (Table 3).

The use of glutamate, succinate and ascorbate in this study makes it possible to ascertain the extent to which the various phosphorylating sites are affected by garlic oil. For example, phosphorylating sites 1, 2 and 3 are active during oxidation of

TABLE 3. EFFECT OF GARLIC OIL ON OXIDATIVE PHOSPHORYLATION IN HEPATIC MITOCHONDRIA OF MICE USING ASCORBATE AS SUBSTRATE*

Concn of garlic oil (μl)	O_2 uptake (μatoms/min per mg protein)	Pi esterified ($\times 10^{-2}$)	P/O ratio	Per cent of control P/O ratio
0 (Control)	2·13 ± 0·17	1·85 ± 0·10	0·87 ± 0·03	100
0·1	2·21 ± 0·09	1·57 ± 0·21	0·71 ± 0·08	82
0·5	2·12 ± 0·05	0·66 ± 0·05	0·31 ± 0·02	36
1·0	2·04 ± 0·08	0·37 ± 0·02	0·18 ± 0·01	21

* Each flask contained phosphate buffer (30 μmoles; pH 7·4), $MgCl_2$ (15 μmoles), EDTA (3 μmoles), ATP (7·5 μmoles), KF (39 μmoles), cytochrome c (0·03 μmole) and 0·5 ml of mitochondrial suspension corresponding to 12 mg protein in 0·075 M sucrose in the main compartment and hexokinase (5 mg), glucose (75 μmoles) and ascorbate (150 μmoles) in the side arm, all in a total fluid volume of 3 ml. The center well contained 0·2 ml of 20% KOH with a small roll of Whatman No. 1 filter paper. Values are means ± S.E. based on four independent determinations. Experimental conditions were as described in Materials and Methods.

glutamate, sites 2 and 3 are functional during oxidation of succinate, and site 3 alone is operative during oxidation of ascorbate. The P/O ratio decreases with increasing concentrations of garlic oil.

The results on the effect of 0·5 μl diallyl disulfide and dipropyl disulfide on oxygen uptake and the P/O ratio using glutamate, succinate and ascorbate are presented in Table 4. Diallyl disulfide is much more inhibitory than dipropyl disulfide. The effect of the former is comparable with that of garlic oil. The data also show that while respiration associated with the oxidation of glutamate was inhibited significantly by both the compounds, respiration associated with succinate and ascorbate remains unaffected (Table 4). Diallyl disulfide inhibited the P/O ratio with glutamate, succinate and ascorbate as substrates by 53, 36 and 59 per cent respectively. Inhibition of the P/O ratio due to dipropyl disulfide during oxidation of glutamate, succinate and ascorbate was 17, 7 and 17 per cent respectively (Table 4).

DISCUSSION

The present studies show that garlic oil is an effective inhibitor of oxidative phosphorylation *in vitro* in hepatic mitochondria of mice. The extent to which respiration is affected by garlic oil varies, depending upon the substrates used. The data show that respiration is more susceptible to the action of garlic oil during the oxidation of glutamate than during the oxidation of succinate and ascorbate. Rotenone, an insecticide of plant origin, has been shown to inhibit the oxidation of glutamate by mitochondria of rat liver and cockroach muscle but not the oxidation of succinate.[13] A similar effect has also been reported for scopafungin, an antibiotic, in rat liver.[14] Phosphorylation, however, is inhibited to a greater extent than respiration with all the three substrates used. It may be inferred that garlic oil acts mainly as an uncoupler of oxidative phosphorylation. It has been shown that 2,4-dinitrophenol (DNP) affects phosphorylating sites 1, 2 and 3.[15] There is, thus, a certain similarity between the effects of DNP and garlic oil.

Diallyl disulfide, the main component of garlic oil, inhibits mitochondrial oxidative phosphorylation to an extent similar to that of garlic oil. Dipropyl disulfide, a substance which is chemically comparable to diallyl disulfide but which lacks its insecticidal activity, does not influence the P/O ratio appreciably. It may be pointed out here that the incorporation of [^{14}C]leucine into proteins of mosquito larvae is reduced by diallyl disulfide but not by dipropyl disulfide.[7]

Many attempts have been made to correlate the toxic action of several commonly used insecticides with their effect on oxidative phosphorylation. DDT has been shown to inhibit oxidative phosphorylation in respiratory particles prepared from houseflies[16] and mosquitoes.[17] DDT and its analogues inhibit the ATP–^{32}P exchange reaction in mosquito sarcosomes.[18]

It may be surmized that: (1) garlic oil inhibits oxidative phosphorylation *in vitro* in hepatic mitochondria of mice; (2) the inhibition and uncoupling affect phosphorylating sites 1, 2 and 3; (3) respiration is more susceptible to the action of garlic oil during the oxidation of glutamate than during the oxidation of succinate or ascorbate; (4) phosphorylation is invariably inhibited to a greater extent than respiration, suggesting that garlic oil acts primarily as an uncoupler of oxidative phosphorylation; and (5) diallyl disulfide, the main component of garlic oil, inhibits oxidative phosphorylation in hepatic mitochondria to the same extent as garlic oil.

TABLE 4. EFFECT OF DIALLYL DISULFIDE AND DIPROPYL DISULFIDE ON OXIDATIVE PHOSPHORYLATION IN HEPATIC MITOCHONDRIA OF MICE USING DIFFERENT SUBSTRATES *

Substrates	Other additions	O_2 uptake (μatoms/min/mg protein)	Pi esterified (μatoms/min/mg protein $\times 10^2$)	P/O ratio	Per cent of control P O ratio
Glutamate	None (control)	1·66 ± 0·11	5·05 ± 0·38	3·04 ± 0·08	100
Glutamate	+ Diallyl disulfide	0·71 ± 0·10	1·03 ± 0·18	1·45 ± 0·09	47
Glutamate	+ Dipropyl disulfide	1·01 ± 0·06	2·55 ± 0·19	2·52 ± 0·11	83
Succinate	None (control)	1·73 ± 0·11	3·22 ± 0·25	1·86 ± 0·05	100
Succinate	+ Diallyl disulfide	1·74 ± 0·16	2·05 ± 0·13	1·20 ± 0·11	65
Succinate	+ Dipropyl disulfide	1·75 ± 0·13	3·02 ± 0·20	1·74 ± 0·14	94
Ascorbate	None (control)	2·13 ± 0·17	1·85 ± 0·10	0·87 ± 0·03	100
Ascorbate	+ Diallyl disulfide	2·15 ± 0·07	0·77 ± 0·09	0·36 ± 0·04	41
Ascorbate	+ Dipropyl disulfide	2·10 ± 0·06	1·50 ± 0·02	0·72 ± 0·02	83

* The concentration of diallyl disulfide and dipropyl disulfide was 0·5 μl. The composition of the incubation medium, for the respective substrates, was as described in Tables 1, 2 and 3. Values are means ± S.E. based on four independent determinations. Experimental conditions were as described in Materials and Methods.

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