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THE UNCOUPLING EFFECT OF *N*-(PHOSPHONOMETHYL)GLYCINE ON ISOLATED RAT LIVER MITOCHONDRIA

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Abstract—The rates of oxygen consumption by rat liver mitochondria, respiring on either succinate, a two-site substrate, or β-hydroxybutyrate, a three-site substrate, and in the presence of varying concentrations of the isopropylamine salt of *N*-(phosphonomethyl) glycine (PMG) have been measured polarographically. The respiratory control ratios of these mitochondria were shown to be significantly reduced, by at least 10 per cent by the addition of 3.95×10^{-5}M PMG. There was a larger decrease in these ratios, up to 50 per cent, as the concentration of the herbicide was raised to 1.25×10^{-3}M. At concentrations ranging from 3.12×10^{-4}M to 1.25×10^{-3}M, PMG restored respiration of mitochondria previously inhibited by oligomycin. Adenosine triphosphatase (ATPase) activity was enhanced by the addition of PMG. In this respect, the maximal increase, 3-fold, was obtained at 6.25×10^{-4}M PMG. These findings suggest that *N*-(phosphonomethyl) glycine uncouples oxidative phosphorylation in isolated rat liver mitochondria.

Very few compounds derived from α-amino acids are able to permeate the lipid bilayer between the outer and the inner mitochondria membrane to produce deleterious effects on the inner mitochondria membrane functions such as coupling of ADP and inorganic phosphate, electron transfer, calcium transport and protein synthesis. Sarcolysine (Merphalan), which is used as an antitumour agent, uncouples mitochondria [1] by an undefined mechanism. Thyroxine acts as an uncoupler of oxidative phosphorylation [2–5] after preincubation with mitochondria, although thyroid hormones exert a direct effect upon mitochondria by stimulating oxygen consumption [6] and mitochondrial protein synthesis [7].

N-phosphonomethyl derivative of glycine (glyphosate) is used as a broad-spectrum and non-selective herbicide [8]. This compound has been shown to increase the rectal temperature of rat after a sublethal dosage of 235 mg *N*-(phosphonomethyl)glycine (PMG)/kg [9]. Several investigations [10–12] have shown that compounds which possess similar hyperthermal effects are able to uncouple mitochondrial oxidative phosphorylation.

In the present work, the *in vitro* effects of *N*-(phosphonomethyl) glycine (PMG) on oxygen consumption and ATPase activity of rat liver mitochondria were studied.

MATERIALS AND METHODS

N-(phosphonomethyl) glycine (isopropylamine salt) was a gift from the Monsanto Company, Brussels, Belgium. Isolation of rat liver mitochondria (RLM) was carried out essentially according to the method of Schneider [13]. Mitochondrial protein was estimated according to Murphy and Keis [14]. Oxygen consumption was measured polarographically by using a Clark-type oxygen electrode (supplied by Yellow Springs Instrument Co. (YS1), Ohio, U.S.A.) linked to a Perkin–Elmer recorder, model 56. Respiratory control ratio of mitochondria was computed as the ratio of the rate of ADP-stimulated respiration (State 3) to that of ADP-limited respiration (State 4) according to Chance and Williams [15]. ATPase activity was measured according to the method described by Bababunmi and Bassir [16].

RESULTS

Figure 1 shows the patterns of oxygen uptake by RLM respiring, in the absence of added ADP and in the

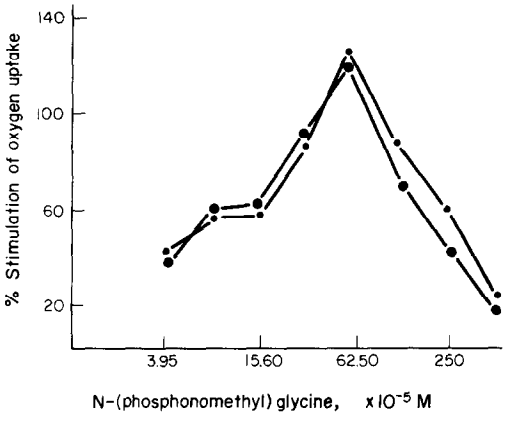

Fig. 1. Effect of *N*-(phosphonomethyl) glycine (PMG) on the rate of oxygen uptake by rat liver mitochondria (4 mg protein) respiring on either succinate, 3.3 mM (●——●) or β-hydroxybutyrate, 3.3 mM (•——•) in the absence of added ADP (respiratory state 4). The reaction vessel (26°) contained as final concentrations 5 mM $MgCl_2$, 5 mM KCl, and 20 mM phosphate buffer, pH 7.4, in a total volume of 3.0 ml. An aliquot of PMG was added 1½ min after the start of the oxidation of the substrate and the difference in the rate of oxygen uptake obtained 1½ min after the addition of PMG was expressed as a percentage of the initial rate of oxygen uptake when substrate alone was being oxidized. Experiments were performed in duplicates for each concentration of PMG and for each mitochondrial sample. At least 10 animals were used.

Table 1. Effect of *N*-(phosphonemethyl) glycine on the respiratory control of isolated rat liver mitochondria respiring on succinate

PMG (M)	Respiratory Control Ratio (a)	(b)	% Reduction
3.95×10^{-5}	4.60 ± 0.20	3.91 ± 0.22	15.0
7.80×10^{-5}	4.39 ± 0.32	3.71 ± 0.26	19.5
1.56×10^{-4}	4.24 ± 0.22	2.80 ± 0.28	32.9
3.12×10^{-4}	4.30 ± 0.26	2.13 ± 0.18	50.0
6.25×10^{-4}	4.10 ± 0.34	1.84 ± 0.21	55.1
1.25×10^{-3}	4.21 ± 0.24	2.71 ± 0.28	55.3
2.50×10^{-3}	4.30 ± 0.28	3.82 ± 0.24	11.2
5.00×10^{-3}	4.56 ± 0.29	2.79 ± 0.23	38.8
10.00×10^{-3}	4.21 ± 0.33	1.47 ± 0.25	65.0

Results are expressed as mean values ± standard deviation for at least five different experiments on each mitochondrial preparation. All results using at least 10 animals were comparable at 95% level of confidence.
PMG = *N*-(phosphomethyl) glycine.
Column (a) No PMG in reaction medium.
Column (b) PMG added to reaction medium.

presence of PMG, on either succinate a two-site substrate or β-hydroxybutyrate, a three-site substrate. By comparing the respiratory rates (millimicroatoms O_2 per min) immediately before and after the addition of PMG, the herbicide caused an enhancement of the rate of oxygen uptake by RLM. The addition of 7.80×10^{-5}M PMG caused the rate of oxygen uptake to increase by about 58 per cent when β-hydroxybutyrate was used as the substrate. The rate was also increased by a factor of 2.17 (117 per cent) when the concentration of the herbicide was increased to 6.25×10^{-4}M.

The extent of these increases decreased when the concentration of PMG was changed (decreased or increased) from 6.25×10^{-4}M (Fig. 1). Similar results were obtained when succinate was used as substrate (Fig. 1). At 6.25×10^{-4}M PMG, the enhancement of the rate of oxygen uptake was of the order of 123 per cent.

Table 2. Effect of *N*-(phosphonomethyl) glycine on the respiratory control of isolated rat liver mitochondria respiring on β-hydroxybutyrate

PMG	Respiratory control ratio (a)	(b)	% Reduction
3.95×10^{-5}	4.50 ± 0.31	4.00 ± 0.28	11.0
7.80×10^{-5}	4.16 ± 0.36	3.41 ± 0.27	18.0
1.56×10^{-4}	4.55 ± 0.30	2.96 ± 0.24	34.9
3.12×10^{-4}	4.48 ± 0.33	2.61 ± 0.21	41.7
6.25×10^{-4}	4.50 ± 0.25	2.10 ± 0.22	53.3
1.25×10^{-3}	4.23 ± 0.31	3.64 ± 0.25	12.7
2.50×10^{-3}	4.00 ± 0.25	3.60 ± 0.26	10.0
5.00×10^{-3}	4.21 ± 0.24	1.30 ± 0.21	69.1
10.00×10^{-3}	4.36 ± 0.27	1.00 ± 0.19	77.0

Results are expressed as mean values ± standard deviation for at least five different experiments on each mitochondrial preparation. All results using at least 10 animals were comparable at 95% level of confidence.
PMG = *N*-(phosphonomethyl) glycine.
Column (a) No PMG in reaction medium.
Column (b) PMG added to reaction medium.

Table 3. Effect of various concentrations of *N*-(phosphonomethyl) glycine on ATPase activity of isolated rat liver mitochondria

PMG (M)	ATPase activity (moles Pi/mg protein/min)
Control	0.91 + 0.05
9.70×10^{-6}	1.11 ± 0.04
1.95×10^{-5}	1.20 ± 0.06
3.90×10^{-5}	1.60 ± 0.05
7.80×10^{-5}	1.81 ± 0.07
1.56×10^{-4}	1.92 ± 0.07
3.12×10^{-4}	2.36 ± 0.09
6.25×10^{-4}	2.91 ± 0.06
1.25×10^{-3}	2.46 ± 0.09
2.50×10^{-3}	1.80 ± 0.04
5.00×10^{-3}	1.00 ± 0.03
10.00×10^{-3}	0.81 ± 0.04
3.00×10^{-4} DNP	10.21 ± 0.63

Results are expressed as mean values ± standard deviation for at least five different experiments on each mitochondrial preparation. All results using at least 10 animals were comparable at 95% level of confidence.
PMG = *N*-(phosphonomethyl) glycine.

Table 1 summarises the effect of PMG on ADP-stimulated oxygen uptake by RLM respiring on succinate. The data obtained show that the additions of varying concentrations of PMG (3.95×10^{-5}M to 2.50×10^{-3}M) resulted in decreases in the respiratory control ratios of RLM. Maximal decreases (50 and 55 per cent) were obtained at concentrations of 3.12×10^{-4} and 6.25×10^{-4}M PMG, respectively. When β-hydroxybutyrate was used as substrate, the pattern of the effect of PMG was similar to that obtained when mitochondria were respiring on succinate (Table 2). Maximal reduction of respiratory control ratio (53 per cent) was obtained at 6.25×10^{-4}M PMG.

The data presented in Table 3 reveal that PMG enhanced ATPase activity. Maximal enhancement (3-fold) was obtained at a concentration of 6.25×10^{-4}M PMG. The enhancement caused by 2,4-dinitrophenol was about 3-fold that of PMG (Table 3).

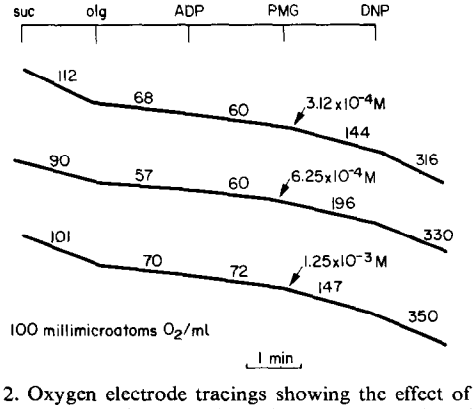

Fig. 2. Oxygen electrode tracings showing the effect of *N*-(phosphonomethyl) glycine (PMG) on oligomycin-inhibited respiration in isolated rat liver mitochondria (RLM, 4 mg protein). Arrows indicate the points of successive additions of succinate (3.3 mM), oligomycin (10 μg) ADP (0.3 mM), PMG and DNP (0.003 mM) in a total volume of 3 ml. Numbers along tracings are the rates of oxygen consumption expressed in millimicroatoms O_2 per min. Temperature 26°.

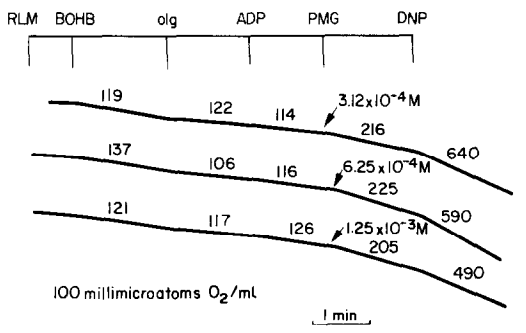

Fig. 3. Oxygen electrode tracings showing the effect of *N*-(phosphonomethyl) glycine (PMG) on oligomycin-inhibited respiration in isolated rat liver mitochondria (RLM). Arrows indicate the points of successive additions of RLM (4 mg protein), β-hydroxbutyrate (3.3 mM), oligomycin (10 μg), ADP (0.3 mM), PMG, and DNP in a total volume of 3 ml. Numbers along the tracings are the rates of oxygen consumption expressed in millimicroatoms O_2 per min. Temperature 26°.

Figure 2 represents the pattern of the effects of oligomycin and PMG on the rate of oxygen consumption by RLM respiring on succinate. When PMG was added at a concentration of 3.12×10^{-4}M, the rate of oxygen uptake was increased to 144 millimicroatoms O_2 per min (Fig. 2). The additions of 6.25×10^{-4}M and 1.25×10^{-3}M PMG also resulted in increases (at least by a factor of 2) in the rate of oxygen uptake by RLM. Similar results were obtained when mitochondria were respiring on β-hydroxybutyrate (Fig. 3).

DISCUSSION

Two major characteristics of uncoupled mitochondria are (a) an enhanced respiration rate and (b) the appearance of ATPase activity [17–19]. Several classes of foreign compounds (xenobiotics) such as the dinitrophenol and hydroxybenzonitrile pesticides [20, 21] are known to uncouple mitochondrial oxidative phosphorylation. Some amino acids or their derivatives, for example sarcolysine [1] and thyroxine [2–5], have been shown to uncouple mitochondrial respiration. Results obtained from experiments with *N*-phosphonomethyl derivative of glycine demonstrate that by using either a 3-site or a 2-site substrate, the herbicide at a concentration of 6.25×10^{-4}M caused maximal reduction of respiratory control ratio by at least 50 per cent (Tables 1 and 2). ATPase activity was also maximally enhanced at 6.25×10^{-4}M PMG. In a manner similar to that of the classical uncoupler, DNP, the herbicide restores the respiration which was inhibited by oligomycin (Figs. 2 and 3). These observations therefore disclose an uncoupling property of *N*-(phosphonomethyl) glycine on mitochondrial respiration.

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REFERENCES

1. T. N. Ramanova, and L. F. Astakhova, *Chem. Abstr.* 1937, 1w. (1971).
2. F. Dickens and D. Salmony, *Biochem. J.* **64**, 645 (1956).
3. H. A. Lardy and F. Feldott, *Ann N.Y. Acad Sci.* **54**, 636 (1951).
4. C. Martins and B. Hess, *Archs Biochem. Biophys.* **33**, 486 (1951).
5. J. H. Park, B. P. Merkwether, and C. R. Park, *Biochim. biophys. Acta* **28**, 662 (1958).
6. M. P. Primack, D. F. Tapley and J. L. Buchanan, *Biochim. biophys. Acta* **244**, 349 (1971).
7. J. L. Buchanan, M. P. Primack, and D. F. Tapley, *Endocrinology* **87**, 993 (1970).
8. Monsanto. Tech Bull. Glyphosate, MON 1139 and 2139 (1971).
9. E. A. Bababunmi, O. O. Olorunsogo, and O. Bassir *Toxic. appl. Pharmac,* in press. (1978).
10. W. Diechmann, W. Machle, K. V. Kitzmiller and G. Thomas. *J. Pharmac. exp. Ther.* **76**, 109 (1942).
11. C. N. Piess, and J. Field, *J. biol. Chem.* **175**, 49 (1948).
12. P. Bufa, D. Carafoli, and U. Muscatello, *Biochem. Pharmac.* **12**, 769 (1963).
13. W. C. Schneider, *J. biol. Chem.* **176**, 259 (1948).
14. J. B. Murphy and M. W. Kies, *Biochim. biophys. Acta* **45**, 328 (1960).
15. B. Chance, and G. R. Williams, *J. biol. Chem.* **227** 383 (1955).
16. E. A. Bababunmi, and O. Bassir. *FEBS Lett.* **26**, 102 (1972).
17. H. A. Lardy and H. Wellman *J. biol. Chem.* **201**, 357 (1953).
18. J. B. Chappel, *Biochem. J.* **90**, 237 (1964).
19. V. H. Parker, *Biochem. J.* **97**, 658 (1965).
20. A. H. Kirby. *Wld Rev. Pest Contr.* **5**, 30 (1966).
21. J. R. Corbett, and J. Goose, *Pest Sci.* **2**, 119 (1971).