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SHORT COMMUNICATIONS

Defective nicotinamide nucleotide transhydrogenase reaction in hepatic mitochondria of *N*-(phosphonomethyl)-glycine treated rats

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Investigations with various preparations of the inner mitochondrial membrane nicotinamide nucleotide transhydrogenases from mammalian tissues have shown that both the rate and extent of reduction of $NADP^+$ by NADH are enhanced by respiratory energy or ATP in an uncoupler sensitive manner [1]. Although this reaction has been shown to give rise to a transmembrane electrochemical gradient in submitochondrial particles as well as in reconstituted liposomes [2, 3], the enzyme probably plays two roles of either generating reducing equivalents (NADH) from NADPH for utilization by the respiratory chain or yielding NADPH from NADH for biosynthetic processes within the cell [1]. There is evidence that a number of chemicals which interfere with mitochondrial oxidative phosphorylation exhibit specific effects on the transhydrogenase reaction. For example, thyroxine, an α-amino acid derived hormone has been shown to uncouple mitochondrial respiration as well as to inhibit the transhydrogenase reaction [4]. Whilst certain chemical compounds, for example, oligomycin, which inhibit coupling have no effect on respiration-driven transhydrogenase reaction [5], 2,4-dinitrophenol, a classical uncoupler of oxidative phosphorylation will inhibit both ATP- and respiration-driven reactions [5]. Although *N*-(phosphonomethyl) glycine (PMG), a broad-spectrum and non-selective herbicide has been shown to uncouple mitochondrial oxidative phosphorylation in hepatic cells of rat liver [6, 7], it is not known whether this glycine-derived herbicide will interfere with energy-linked transhydrogenase reactions in mitochondria isolated from rats which received various doses of the herbicide.

Five groups each of five female albino rats (Wistar strain) obtained from pathogen-free colonies of the Preclinical Animal Breeding House, College of Medicine, University of Ibadan, Ibadan, Nigeria and weighing approximately 250 g per rat were kept in separate cages where they were fed with purina chow and water *ad lib*. The first four groups of animals received by i.p. injection 30, 60, 120 and 240 mg PMG/kg body weight, respectively, whilst the fifth group of rats were given comparable volumes of doubly distilled sterile water which was the vehicle used for injecting the herbicide (a gift from Monsanto Company, Brussels, Belgium). Mitochondrial fractions of liver homogenates of the animals were prepared 5 hr after PMG treatment according to a modification of the method described by Schneider [8]. All isolation procedures were carried out in the cold at 0–4°. Ten per cent homogenates (in ice-cold 0.25 M sucrose and 0.1 M Tris–HCl, pH 7.4) were immediately centrifuged at 1200 rpm for 10 min in an MSE angle 13 refrigerated high speed centrifuge. The mitochondrial pellet which was sedimented at 10,000 rpm for 10 min was resuspended in not less than 2 ml of the sucrose–Tris medium. Mitochondrial protein was estimated according to Murphy and Kies [9]. Nicotinamide nucleotide transhydrogenase was followed by fixing aliquots of the reaction mixture at suitable time intervals and determining the concentration of NADH and NADPH by specific enzymic methods as described by Lee and Ernster [10]. The reaction medium contained in a final volume of 20 ml, 200 mM sucrose. Five M Tris–HCl pH 7.5, 5 mM sodium succinate or 0.3 mM Na_2ATP, 3 μM rotenone, 10 mM $MgSO_4$, 88 μM NADH, 86 μM $NADP^+$ and aliquots of PMG. Mitochondrial fraction equivalent to 6.5 mg protein was added to initiate the reaction at 30°. To stop the reaction, 2 ml each of the reaction medium was transferred into 0.1 ml of 2 M KOH at 2 min intervals for the first 10 min and subsequently at 5 min intervals for the next 20 min of the reaction. Each quenched reaction medium was diluted with 2 ml each of 20 mM sucrose and 50 μM Tris–HCl (pH 7.5) and neutralized by the addition of 1 M acetic acid (pH 7.5–8.0). For NADH estimation 1 mM pyruvate and 20 μg lactate dehydrogenase were added to 3 ml of the reaction medium and the absorbance measured at 340 nm. For NADPH estimation, 1 mM α-ketoglutarate, 3 μM NH_4Cl and 20 μg glutamate dehydrogenase were added to the reaction medium and 1–2 min absorbance was read at the same wavelength within at least 1–2 min. All chemicals were purchased from Sigma Chemical Co., (St. Louis, MO). Depending on the state and source of transhydrogenase, its activity can be estimated in four different ways [1]. The respiratory control ratio of the mitochondrial samples used for this investigation was not less than 3.5 when succinate was used as substrate.

The results presented in Figs. 1 and 2 indicate that whilst there was no effect on either the ATP- or respiration-powered transhydrogenase reaction at PMG dose < 30 mg/kg, these reactions were inhibited by at least 11.35 per cent at doses > 60 mg/kg. The extent of inhibition

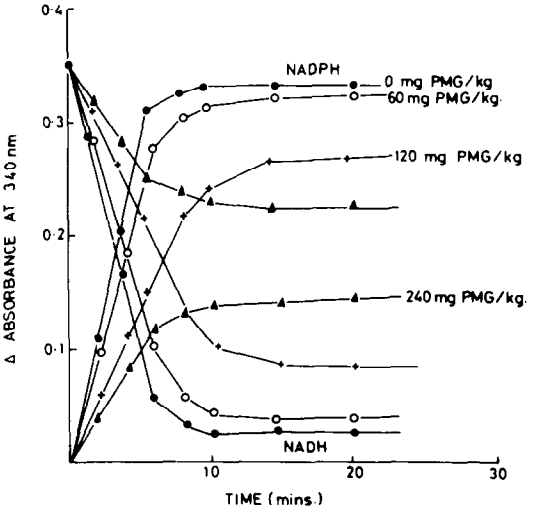

Fig. 1. Pattern of respiration-driven nicotinamide nucleotide transhydrogenase reaction in intact mitochondrial fraction isolated from the livers of rats 5 hr after intraperitoneal dosing with *N*-(phosphonomethyl) glycine (PMG). Reaction medium same as described in text.

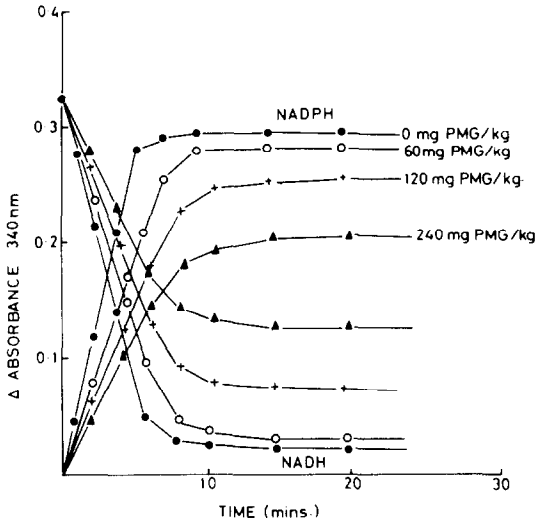

Fig. 2. Pattern of ATP-driven nicotinamide nucleotide transhydrogenase reaction in intact mitochondria fraction isolated from the livers of rats 5 hr after intraperitoneal dosing with *N*-(phosphonomethyl) glycine (PMG). Reaction medium same as described in text except that 0.3 mM ATP was added in place of 5 mM sodium succinate.

increased as the dose of the herbicide was raised to 240 mg PMG/kg which gave maximal inhibitions of 49 and 58% respectively for the ATP- and respiration-driven reactions. The levels of NADPH formed, 10 min after the start of the reactions decreased by at least 17.6% and 33.4% respectively at 120 mg PMG/kg and 240 mg PMG/kg dosage levels when compared to controls. These results suggest that like 2,4-dinitrophenol, a classical uncoupler, PMG induces a defect in the energy-linked nicotinamide nucleotide transhydrogenase reaction.

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The effect of spironolactone pretreatment on digitoxin metabolism in isolated hepatocytes from male and female rats

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In a previous paper [1], we reported a preliminary result for digitoxin (Dt3*) metabolism in isolated rat hepatocyte preparations. In order to establish the validity of this system for the study of Dt3 metabolism, the present study was designed to test the effect of spironolactone (SP) pretreatment on Dt3 metabolism in isolated rat hepatocyte preparations obtained from female and male rats.

[^{3}H] Dt3 (10.9 Ci/mmole, generally labeled) and [1α, 2α-^{3}H]cholesterol (57 Ci/mole) were purchased from New England Nuclear (Boston, MA). Unlabeled Dt3 and Dg3 were from Merck (Darmstadt, West Germany), Dt2, Dt1, Dt0, Dg2, Dg1 and Dg0 from Boehringer Mannheim (Mannheim, West Germany), collagenase (type I) from Sigma (St. Louis, MO). Thirteen-week-old Wistar derived rats of both sexes were used.

Aldactone A tablets (Dainihonseiyaku, Osaka, Japan) were ground into powder and suspended in distilled water. This suspension was given orally (10 mg/100 g body wt as a SP dose) twice daily for 4 consecutive days. About 18 to 20 hr after the last dose, rats were used for the preparation of isolated hepatocytes.

The isolation of rat hepatocytes was performed according to the method described previously [1]. After preparation of isolated hepatocytes, the cells were resuspended in Waymouth MB 752/1 medium containing HEPES, alanine and serine. A [^{3}H] Dt3 DMSO solution with various amounts of unlabeled Dt3 was added to the cell suspension. The range of the concentration of Dt3 in the medium was from

* Abbreviations used: Dt3, digitoxin; Dt2, digitoxigenin bis-digitoxoside; Dt1, digitoxigenin *mono*-digitoxiside; Dt0, digitoxigenin; Dg3, digoxin; Dg2, digoxigenin *bis*-digitoxoside; Dg1, digoxigenin *mono*-digitoxoside; Dg0, digoxigenin; DMSO, dimethyl sulfoxide; SP, spironolactone.