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RAPID COMMUNICATIONS

HEXACHLOROBENZENE-INDUCED OXYGEN ACTIVATION BY MOUSE LIVER MICROSOMES: COMPARISON WITH PHENOBARBITONE AND 20-METHYLCHOLANTHRENE

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INTRODUCTION

Administration of the porphyrogenic chemical hexachlorobenzene (HCB) to male C57BL/6 mice increases the NADPH-stimulated production of reactive oxygen species (ROS) by liver microsomal fractions [1]. ROS have been proposed to be important in the pathogenesis of the porphyria caused by polyhalogenated aromatic hydrocarbons [2-4], though stimulation by HCB of ROS production is independent of iron pretreatment [1], a prerequisite for HCB-porphyria in mice [5]. ROS are produced during the reactions catalysed by the cytochromes P-450, and may be released during uncoupling of these reactions [6]. Here we compare the effectiveness of HCB, and two other cytochrome P-450 inducers, phenobarbitone (PB) and 20-methylcholanthrene (MC), as stimulators of ROS production.

MATERIALS AND METHODS

Male C57BL/6 mice (wt 15-25 g) were injected intraperitoneally with HCB (organic analytical standard, BDH Chemicals, Poole, Dorset, U.K.) (200 mg/kg in 0.5 ml corn oil), with MC (Sigma Chemical Co., Poole, Dorset, U.K.) (125 mg/kg in 0.5 ml corn oil), or with sodium phenobarbitone (McCarthy Laboratories, Romford, Essex, U.K.) (80 mg/kg in 0.2 ml saline on 3 successive days). Control animals received vehicle only. Time in days was measured from the last injection.

Mice were killed by cervical dislocation. Livers were removed and homogenised in 20 mM tris/HCl containing 0.25 M sucrose. Homogenates were centrifuged (15000 g, 15 min) and the supernatant (microsomal fraction) was used for analysis.

ROS production was measured as lucigenin-enhanced chemiluminescence (CL) [7]. 0.01 ml of 8 nM lucigenin (bis-N-methylacridinium nitrate, Sigma Chemical Co., Poole, Dorset, U.K.) in water was added to microsomal fraction (0.05 ml) in 66 mM tris/HCl pH 7.4 containing 0.1% NaN_3 (0.45 ml). Tubes were placed in a Berthold Biolumat LB95000T luminometer (chamber temperature 37°C) and the reaction initiated by adding 0.1 ml of 2.5 mg/ml NADPH (Sigma Chemical Co., Poole Dorset, U.K.). Luminescence counts were measured for 10 min. Cytochrome P-450 was measured in homogenates [8].

RESULTS AND DISCUSSION

HCB produced a sustained increase in ROS production which reached a maximum at about 8 days and then declined to control levels by day 32 (Fig. 1). A similar pattern was obtained when

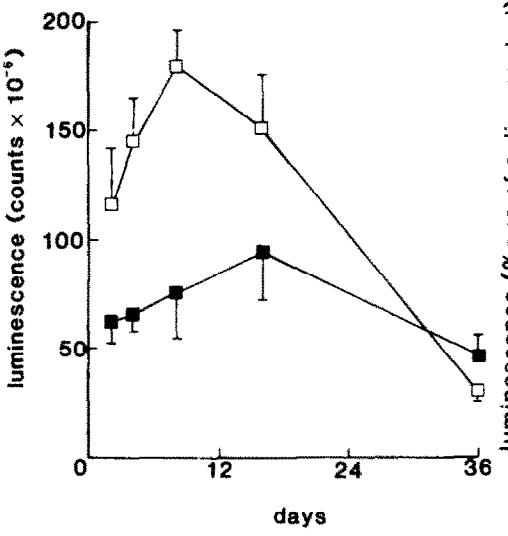

Fig. 1. NADPH-stimulated CL in mice given HCB (□) or vehicle (■). Means and SEM for 4 animals are shown.

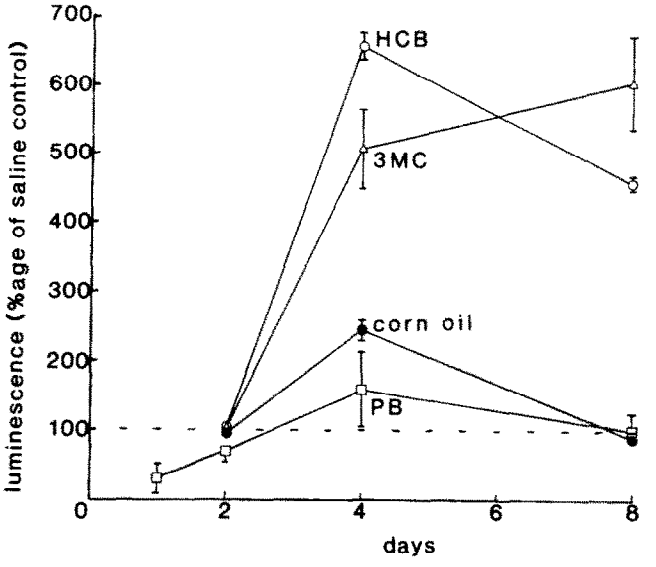

Fig. 2. NADPH-stimulated CL in mice given PB, MC, HCB or vehicle. Results are percentages of mean for saline-treated controls and are means and ranges for groups of 2 mice.

mice were pre-treated with iron dextran (12.5 mg iron/animal) [5] and subsequently developed porphyria at about the time that NADPH-dependent CL returned to the control level. In all experiments, corn oil produced a small increase in CL, the cause of which is uncertain. MC stimulated ROS production in a similar manner to HCB (Fig. 2). In this experiment, there was no increase in CL 2 days after injection, but both HCB and MC caused greatly increased CL on days 4 and 8. PB produced no significant increase in CL at any time point. Cytochrome P-450 concentrations on days 2 and 4 were increased 2.6 to 3.4-fold by MC and PB and 1.5-fold by HCB. HCB is a "mixed-type" inducer of cytochrome P-450, inducing both the PB- and MC-inducible forms [9]. Our results suggest that the form of P-450 responsible for increased NADPH- stimulated lucigenin-enhanced CL is induced by MC. ROS production, like induction of MC-type cytochrome P-450s [2-5], may be a prerequisite for the development of HCB-porphyria but other factors, in addition to iron, are also likely to be involved.

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