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EFFECTS OF 3-METHYLCHOLANTHRENE ON OXIDIZED NICOTINAMIDE-ADENINE DINUCLEOTIDE PHOSPHATE-DEPENDENT DEHYDROGENASES AND SELECTED METABOLITES IN PERFUSED RAT LIVER

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Abstract—Treatment of adult rats with 3-methylcholanthrene over a 3-day period produced significant decreases in hepatic ATP concentrations and elevated the activities of 6-phosphogluconate dehydrogenase and malic enzyme. The decline in ATP was accompanied by a decrease in total adenine nucleotides; however, ATP/ADP ratios were essentially the same in livers of normal and 3-methylcholanthrene-treated rats, and no significant changes were noted in glycolytic and citric acid cycle intermediates. Thus, hepatic energy metabolism does not appear to be altered grossly after 3-methylcholanthrene treatment. $NADP^+$/NADPH ratios calculated from substrates assumed to be in near equilibrium with malic enzyme, isocitrate dehydrogenase and 6-phosphogluconate dehydrogenase increased after 3-methylcholanthrene treatment; these changes were accompanied by significant increases in the activity of malic enzyme and 6-phosphogluconate dehydrogenase. Oxidation of hepatic NADPH and elevation of activities of several NADPH-generating enzymes by 3-methylcholanthrene indicates that this agent which induces components of the mixed-function oxidase system also elevates the capacity of the liver to form NADPH.

The effects of inducing agents, such as phenobarbital, on the activities of the mixed-function oxidase system and UDP glucuronyl transferase involved in glucuronidation are well documented [1, 2]; however, relatively little is known about the effects of these agents on other pathways of intermediary metabolism. Consideration of the actions of inducing agents on other enzyme systems, such as those which supply NADPH and activated substrates for conjugation, is especially important, since the supply of these cofactors may be rate-limiting in intact cells [3, 4]. Phenobarbital has been shown to alter the capacity of the liver to form NADPH as well as to induce components of the mixed-function oxidase system. Activities of glucose-6-phosphate dehydrogenase [5] and malic enzyme were elevated in livers of rats pretreated with phenobarbital [6, 7]. In addition, phenobarbital pretreatment produced a more oxidized state of the NADPH redox system and lowered the intracellular concentrations of ATP [7]. These latter effects could contribute to enhanced generation of NADPH in the intact liver, since the major NADPH-generating enzymes are strongly regulated by the $NADP^+$/NADPH ratio [8] and inhibited by ATP [9, 10].

3-Methylcholanthrene is a carcinogen which induces the activity of aryl hydrocarbon hydroxylase [1], an NADPH-dependent mono-oxygenase system with forms of cytochrome P-450 different from those induced by phenobarbital [11, 12]. Further, phenobarbital, but not 3-methylcholanthrene, induces cytochrome P-450 reductase. The present experiments were designed to examine whether 3-methylcholanthrene can alter the capacity of the liver to form NADPH.

MATERIALS AND METHODS

Animals. Female Sprague–Dawley rats received 3-methylcholanthrene (20 mg/kg, i.p.) or an equivalent volume of the corn oil vehicle for 3 days prior to perfusion experiments. This protocol has been shown to produce maximal induction of the aryl hydrocarbon hydroxylase (EC 1.14.1.1) [13].

Liver perfusion. Details of the perfusion technique have been described elsewhere [14]. Briefly, livers were perfused with Krebs–Henseleit bicarbonate buffer [15], pH 7.4, saturated with an oxygen–carbon dioxide mixture (95 : 5) in a non-recirculating system. The fluid (37°) was pumped via a cannula in the vena cava through the liver and past a teflon-shielded oxygen electrode. Rates of oxygen uptake were routinely calculated from the liver weight, flow rate, and the arterial–venous oxygen concentration difference as a measure of tissue viability.

Metabolite and enzyme determinations. Metabolites were measured in $HClO_4$ extracts of livers that had been freeze-clamped with tongs chilled in liquid nitrogen [16]. Samples of frozen liver weighing about 200 mg were powdered and extracted with 0.3 M

$HClO_4$ as described previously [17]. The protein-free extracts were neutralized with 2 M $KHCO_3$ and stored at −80° until assayed for metabolites.

Intermediates of the Embden–Meyerhoff pathway were measured enzymatically by fluorometric procedures described by Lowry and Passonneau [18]. Intermediates of the tricarboxylic acid cycle were measured by the methods of Goldberg *et al.* [19]. Cytoplasmic $NADP^+$/NADPH ratios were calculated as described previously [7].

Activities of glucose-6-phosphate dehydrogenase (EC 1.1.1.49), 6-phosphogluconate dehydrogenase (EC 1.1.1.43), malic enzyme (EC 1.1.1.40), isocitrate dehydrogenase (EC 1.1.1.42) and glutathione reductase (EC 1.6.4.2) were measured in liver homogenates, as described previously [7, 10].

RESULTS

Pretreatment of rats with 3-methylcholanthrene for 3 days enhanced the activities of several $NADP^+$-dependent dehydrogenases and altered the steady-state concentrations of several metabolites. The activities of 6-phosphogluconate dehydrogenase and malic enzyme were increased 18 and 64 per cent respectively (Table 1). In contrast, the activities of isocitrate dehydrogenase, glutathione reductase and glucose-6-phosphate dehydrogenase were not altered significantly by 3-methylcholanthrene treatment. Elevated activities of hepatic 6-phosphogluconate dehydrogenase, malic enzyme and glucose-6-phosphate dehydrogenase have been noted previously in rats treated with 3-methylcholanthrene [20].

Pretreatment of rats with 3-methylcholanthrene caused both total adenine nucleotides and ATP levels to decline about 20 per cent (Table 2); however, concentrations of ADP and AMP, ATP/ADP ratios, and the energy charge were not altered significantly.

$NADP^+$/NADPH ratios, calculated from substrates assumed to be in near equilibrium with malic enzyme, isocitrate dehydrogenase and 6-phosphogluconate dehydrogenase, tended to increase following treatment with 3-methylcholanthrene (i.e. the NADPH redox state was more oxidized) (Table 3).

Alterations in steady-state concentrations of

Table 1. Enzyme activities in homogenates of livers from normal and 3-methylcholanthrene-treated rats*

Treatment	Isocitrate dehydrogenase	Glutathione reductase	6-Phosphogluconate dehydrogenase	Glucose-6-phosphate dehydrogenase	Malic enzyme
			(μmoles/min/g protein)		
Normal (4)	95.0 ± 1.9	16.0 ± 1.5	10.4 ± 0.8	5.7 ± 0.9	5.5 ± 0.1
3-Methylcholanthrene (5)	99.3 ± 1.2	15.7 ± 0.3	12.3 ± 0.3†	8.1 ± 1.1	9.0 ± 0.8‡

* Enzyme activities were measured in homogenates of whole liver. Values are averages ± S.E.M. from the number of livers indicated in parentheses.
† $P < 0.05$.
‡ $P < 0.01$.

Table 2. Adenine nucleotides in perfused livers from normal and 3-methylcholanthrene-treated rats*

Treatment	ATP	ADP	AMP	Total adenine phosphates	ATP:ADP
			(μmoles/kg wet wt)		
Normal (9)	2238 ± 94	673 ± 23	152 ± 16	2995 ± 76	3.26 ± 0.17
3-Methylcholanthrene (10)	1784 ± 57†	614 ± 29	142 ± 22	2510 ± 75‡	2.95 ± 0.17

* Values are averages ± S.E.M. obtained from the number of livers shown in parentheses.
† $P < 0.05$ (3-methylcholanthrene vs normal).
‡ $P < 0.01$.

Table 3. $NADP^+$:NADPH ratios in perfused livers from 3-methylcholanthrene-treated rats*

Treatment	Malic enzyme	Isocitrate dehydrogenase	6-Phosphogluconate dehydrogenase
None	1.95 ± 0.41	4.31 ± 0.36	3.23 ± 0.94
3-Methylcholanthrene	2.70 ± 0.35	5.35 ± 0.64	5.87 ± 1.64

* Values were calculated from measured concentrations of malate and pyruvate (malic enzyme), α-ketoglutarate and isocitrate (isocitrate dehydrogenase), and ribulose-5-phosphate and 6-phosphogluconate (6-phosphogluconate dehydrogenase), and are averages ± S.E.M. Ratios were calculated from measured concentrations of substrates and equilibrium constants for each of the three dehydrogenases [21].

Table 4. Pentose phosphate pathway intermediates in normal and 3-methylcholanthrene-treated rats*

Treatment	6-Phosphogluconate	Ribulose-5-phosphate	Xylulose-5-phosphate
		(μmoles/kg wet wt)	
Normal (9)	3.82 ± 0.31	16.71 ± 4.99	7.30 ± 3.98
3-Methylcholanthrene (10)	4.89 ± 0.58	36.21 ± 8.28†	14.27 ± 4.27

* Values are averages ± S.E.M. of the same livers shown in Table 2.
† $P < 0.10$.

Table 5. Citric acid cycle intermediates in perfused livers from normal and 3-methylcholanthrene-treated rats*

Treatment	Citrate	Isocitrate	α-Ketoglutarate	Malate
		(μmoles/kg wet wt)		
Normal (9)	95.46 ± 16.17	6.14 ± 0.62	267.42 ± 8.64	87.38 ± 8.41
3-Methylcholanthrene (10)	79.09 ± 10.41	5.05 ± 0.66	247.13 ± 19.52	83.46 ± 6.93

* Values are averages ± S.E.M. of the same livers shown in Table 2.

metabolites measured in livers from 3-methylcholanthrene-treated rats suggest that the carcinogen enhanced metabolic flux via the pentose phosphate pathway. Data in Table 4 compare concentrations of 6-phosphogluconate, ribulose-5-phosphate and xylulose-5-phosphate in livers of normal and 3-methylcholanthrene-treated rats. Concentrations of xylulose-5-phosphate and ribulose-5-phosphate tended to increase ($P < 0.1$), while 6-phosphogluconate concentrations were unchanged. In contrast, concentrations of a number of key intermediates in the citric acid cycle, including citrate, isocitrate, α-ketoglutarate and malate, were unaffected by 3-methylcholanthrene treatment (Table 5).

DISCUSSION

In addition to the well-documented induction of microsomal cytochrome P-448 [16], data presented here indicate that 3-methylcholanthrene also enhances the capacity of the liver to produce NADPH. This action occurs via three actions of the carcinogen: (1) increasing the activities of several NADPH-generating enzymes, (2) lowering of hepatic ATP concentrations, and (3) oxidation of intracellular NADPH. The decline in ATP produced by 3-methylcholanthrene could theoretically produce deinhibition of glucose-6-phosphate dehydrogenase which has a K_i for ATP of 1.85 mM [9, 10], a value which is comparable to ATP concentrations noted in the intact liver (Table 2). This reduction of ATP concentrations probably does not deinhibit other NADPH-generating enzymes, since the K_i of ATP for malic enzyme, 6-phosphogluconate dehydrogenase and isocitrate dehydrogenase are 0.009, 0.114 and 0.181 mM, respectively [9, 10]. These values are significantly lower than ATP concentrations noted in control or 3-methylcholanthrene-treated livers. On the other hand, since free ATP is undoubtedly lower than total concentrations measured in tissue extracts, deinhibition of the latter three enzymes cannot be ruled out.

Oxidation of the intracellular $NADP^+$/NADPH redox system would be expected to stimulate metabolism of hexose phosphates via the pentose pathway. $NADP^+$/NADPH ratios calculated from substrates assumed to be in near equilibrium with dehydrogenases in rat liver under normal physiological conditions indicate that the two dehydrogenases of the pentose phosphate pathway are over 90 per cent inhibited [8]. Moreover, slight increases in $NADP^+$/NADPH ratios have been shown to result in relatively large increases in the activity of 6-phosphogluconate dehydrogenase *in vitro* [7]. Increases in ribulose-5-phosphate and xylulose-5-phosphate measured in livers of 3-methylcholanthrene-treated rats (Table 4) support the hypothesis that metabolism via the pentose pathway was enhanced in this tissue by carcinogen pretreatment.

The experiments presented suggest that 3-methylcholanthrene produces a metabolic state in hepatocytes geared toward rapid NADPH generation. The capacity of 3-methylcholanthrene to enhance NADPH formation in rat liver appears to involve both acute and chronic effects. Induction of the two $NADP^+$-dependent dehydrogenases (6-phosphogluconate dehydrogenase and malic enzyme; Table 1) by 3-methylcholanthrene would be expected to produce long-term effects, whereas alterations in steady-state concentrations of ATP and substrates which supply reducing equivalents are subject to other metabolic events which may rapidly alter this action of 3-methylcholanthrene [10]. It has been shown that rates of mixed-function oxidation in the perfused rat liver and isolated hepatocytes are rate-limited by the supply of NADPH [3, 22, 23]. Thus, increased biosynthesis of NADPH as well as increased cytochrome P-448 content should be taken into account to explain increased rates of polycyclic aromatic hydrocarbon oxidation in intact livers of 3-methylcholanthrene-treated animals. Further study is required to determine whether alteration of the cofactor supply is unique to 3-methylcholanthrene and phenobarbital, or is a general phenomenon that occurs with all compounds that induce mixed-function oxidation.

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