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Effect of methylcholanthrene on biosynthesis and metabolism of bile acids

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THE MICROSOMAL fraction of rat liver homogenate contains NADPH-dependent monooxygenases that catalyze hydroxylations of fatty acids, steroids and a large number of foreign compounds. In many of these reactions the oxygen has been shown to be activated by reduced cytochrome P-450.[1] The reduction of cytochrome P-450 by NADPH is mediated through an electron transport chain including a flavoprotein, NADPH-cytochrome *c* reductase.[1] Many cytochrome-P-450 dependent hydroxylations are stimulated by the administration of different foreign compounds. These inducers can be classified into two groups.[2,3] One group of inducers, to which phenobarbital belongs, stimulates hydroxylation of a large number of compounds including steroids. The other group of inducers, to which 3-methylcholanthrene belongs, stimulates hydroxylation of only a limited number of compounds including many polycyclic hydrocarbons.

Several hydroxylations are involved in the biosynthesis and metabolism of bile acids.[4] The effect of phenobarbital on these hydroxylations was studied recently.[5,6] Phenobarbital was found to stimulate 6β-hydroxylation of taurochenodeoxycholic acid* and of lithocholic acid as well as 7α-hydroxylation of taurodeoxycholic acid. The 7α-hydroxylation of cholesterol and the 12α-hydroxylation of 7α, 12α-dihydroxycholest-4-en-3-one were unaffected or inhibited. Kuntzman *et al.*[7] have reported recently that administration of methylcholanthrene to rats stimulates specifically 7α-hydroxylation of testosterone. In view of this finding it appeared of interest to examine the influence of methylcholanthrene on hydroxylations involved in the biosynthesis and metabolism of bile acids. The present report describes the results of such an investigation.

Methods

[4-^{14}C] Cholesterol (145 μc/mg) was obtained from the Radiochemical Centre, Amersham, England and was purified by chromatography on a column of aluminium oxide, grade III (Woelm, Eschwege, Germany). [7β-^{3}H] Cholest-5-ene-3β,7α-diol (10 μc/mg) and [6β-^{3}H] 7α-hydroxycholest-4-en-3-one (12·5 μc/mg) were prepared as described in previous reports from this laboratory.[8,9] Tritium-labeled taurodeoxycholic acid (8·3 μc/mg) and taurochenodeoxycholic acid (4·7 μc/mg) were prepared from tritium-labeled deoxycholic acid and chenodeoxycholic acid as described by Norman.[10] [24-^{14}C] Lithocholic acid (5 μc/mg) was obtained from New England Nuclear Corp. Boston, Mass. Tritium-labeled 3,4-benzpyrene (Radiochemical Centre) was diluted with unlabeled material to give material with a specific radioactivity of 3·3 μc/mg. Prior to use it was purified by thin layer chromatography in darkness with hexane as solvent. Unlabeled 3,4-benzpyrene, NAD and NADPH were obtained from Sigma Chemical Co., St. Louis, Mo. and 3-methylcholanthrene from Th. Schuchardt, Munchen, Germany.

White male rats of the Sprague–Dawley strain, weighing about 150 g, were injected intraperitoneally daily for 3 days with 3-methylcholanthrene, 25 mg/kg in 1 ml of corn oil. Control rats were injected with 1 ml of corn oil. Preparation and fractionation of liver homogenates (20%, w/v) were performed in a modified Bucher medium, pH 7·4, as described previously.[5] Protein was determined with a micro-Kjeldahl technique.[11] Cholesterol in the 20,000 *g* supernatant fluid was determined as described by Hanel and Dam.[12]

[4-^{14}C] Cholesterol, 10 μg in 50 μl of acetone, was incubated for 60 min with a mixture of 3 ml of 20,000 *g* supernatant fluid and 2 ml of Bucher medium. [7β-^{3}H] Cholest-5-ene-3β, 7α-diol, 40 μg in

*The following systematic names are given to bile acids referred to by trivial names: chenodeoxycholic acid, 3α, 7α-dihydroxy-5β-cholanoic acid; deoxycholic acid, 3α, 12α-dihydroxy-5β-cholanoic acid lithocholic acid, 3α-hydroxy-5β-cholanoic acid.

50 μl of acetone, was incubated for 20 min with 1 ml of microsomal suspension, 2 ml of Bucher medium and 1·3 μmoles of NAD. [6β-^{3}H] 7α-Hydroxycholest-4-en-3-one, 80 μg in 50 μl of acetone, was incubated for 10 min with 3 ml of microsomal suspension and 3 μmoles of NADPH. Tritium-labeled taurodeoxycholic acid, 200 μg in 0·2 ml of Bucher medium and taurochenodeoxycholic acid, 150 μg in 0·2 ml of Bucher medium, were incubated for 20 min with 1 ml of microsomal suspension, 2 ml of Bucher medium and 3 μmoles of NADPH. [24-^{14}C] Lithocholic acid, 100 μg of the sodium salt dissolved in 0·5 ml of 25% (v/v) aqueous ethanol, was incubated for 20 min with 2 ml of microsomal suspension, 1 ml of Bucher medium and 3 μmoles of NADPH. All incubations were run at 37°. The different incubation mixtures were worked up and analyzed as described in previous communications.[5,6] Hydroxylation of 3,4-benzpyrene was assayed essentially as described by Silverman and Talalay.[13] Tritium-labeled 3,4-benzpyrene, 50 μg in 50 μl of acetone, was incubated in darkness for 15 min at 37° with 0·5 ml of microsomal suspension (0·1 ml when methylcholanthrene-treated rats were used), 1 ml of Bucher medium and 3 μmoles of NADPH. Incubation was terminated by the addition of 3·5 ml of 0·25 M potassium hydroxide in 50% (v/v) aqueous ethanol. Hexane, 10 ml, was added and the mixture was shaken in darkness for 30 min at room temperature. The phases were allowed to settle for 10 min and 3 ml of the hexane phase was transferred into vials to which were added 15 ml of scintillating solution (4 g of 2,5-diphenyloxazole and 50 mg of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene in 1 l. of toluene). Conversion into hydroxylated products was estimated as the difference between the amount of radioactivity incubated and that recovered in the hexane phase. Determinations were made in triplicate. No conversion was observed if enzyme was omitted from the incubation mixture.

Radioactivity was determined with a Packard Tri-Carb scintillation spectrometer or with a methane gas flow counter.

Results

Thin layer chromatography of extracts of incubations with the different neutral steroids showed that the patterns of products were the same in control rats and in methylcholanthrene-treated rats and that they were very similar to those observed in a previous investigation.[5] The total conversion of the substrates was about the same in the two groups of animals (Fig. 1 A, B and C). The rate of 7α-hydroxylation of taurodeoxycholic acid was not affected by methylcholanthrene treatment (Fig. 1D). The 6β-hydroxylation of taurochenodeoxycholic acid and lithocholic acid was less efficient in methylcholanthrene-treated rats than in control rats (Fig. 1E and F). On an average, the rates of 6β-hydroxylation of taurochenodeoxycholic acid and lithocholic acid were respectively 2·2 and 1·8 times faster in control rats than in methylcholanthrene-treated rats. The extent of formation of hydroxylated derivatives of 3,4-benzpyrene was on an average 6·8 times greater in methylcholanthrene-treated rats than in control rats (Fig. 1G), which is in agreement with the results reported by Silverman and Talalay.[13]

Discussion

The results of the present investigation show that administration of methylcholanthrene has no stimulatory effect on hydroxylations involved in the biosynthesis and metabolism of bile acids. The hydroxylations involved in the biosynthesis of bile acids have been previously shown to be largely unaffected by phenobarbital treatment.[5] The mechanisms of these hydroxylations are not known but it may be concluded that neither phenobarbital nor methylcholanthrene greatly affects any rate-limiting component of these hydroxylases. The hydroxylations involved in the interconversion of bile acids have been shown to be stimulated by phenobarbital treatment and evidence has been presented to indicate that the 6β-hydroxylation of taurochenodeoxycholic acid and lithocholic acid involves the participation of a cytochrome P-450.[6,14,15] The lack of stimulation of 6β-hydroxylation by methylcholanthrene could be interpreted as evidence in favor of the concept that more than one cytochrome P-450 are present in liver microsomes. It has been suggested that methylcholanthrene stimulates the formation of a cytochrome P-450 that is different from that formed upon administration of phenobarbital.[8] No conclusive evidence for the participation of a cytochrome P-450 in the 7α-hydroxylation of taurodeoxycholic acid has been presented. It is interesting that this reaction is not affected by methylcholanthrene treatment. This contrasts with the marked stimulation by methylcholanthrene of the 7α-hydroxylation of testosterone.[7]

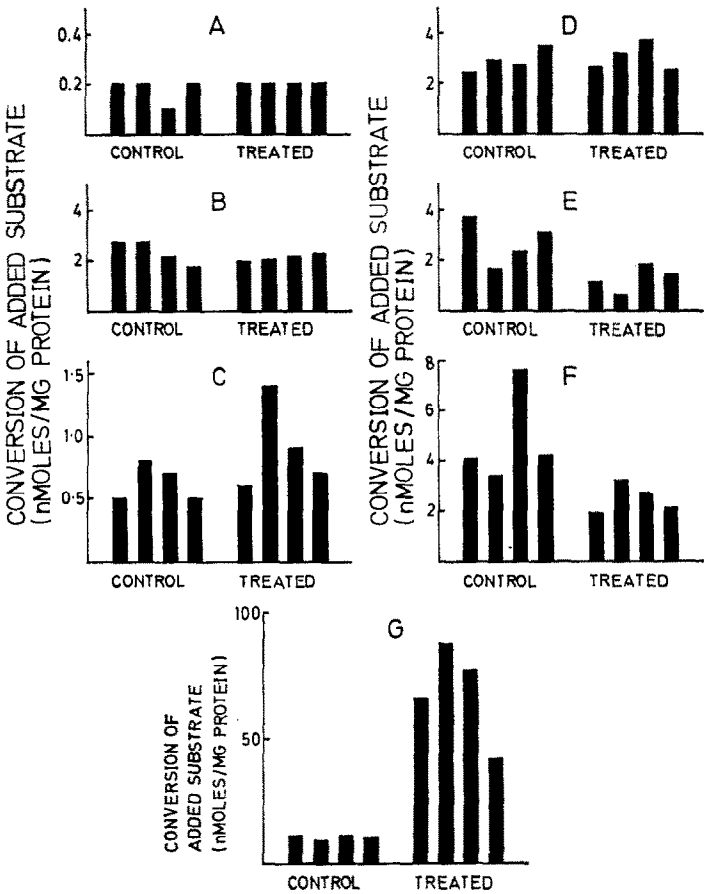

FIG. 1. Effect of methylcholanthrene treatment on reactions in the biosynthesis and metabolism of bile acids and on the metabolism of 3,4-benzpyrene. Control, control rat; treated, 3-methylcholanthrene-treated rat. A, 7α- hydroxylation of cholesterol; B, conversion of cholest-5-ene-3β, 7α-diol into 7α-hydroxycholest-4-en-3-one; C, 12α-hydroxylation of 7α-hydroxycholest-4-en-3-one; D, 7α-hydroxylation of taurodeoxycholic acid; E, 6β-hydroxylation of taurochenodeoxycholic acid; F, 6β-hydroxylation of lithocholic acid; G, conversion of 3,4-benzpyrene into products not extractable with hexane from an alkaline phase containing ethanol. The values listed below each other were obtained with liver homogenate from the same animal.

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