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THE EFFECT OF 3-METHYLCHOLANTHRENE ON URINARY 6β-HYDROXYCORTISOL EXCRETION AND HEPATIC ENZYME ACTIVITY IN THE MARMOSET MONKEY (*CALLITHRIX JACCHUS*)

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Abstract—The effect of 3-methylcholanthrene treatment on the urinary excretion of 6β-hydroxycortisol and hepatic enzyme activity was investigated in the marmoset monkey (*Callithrix jacchus*). 3-Methylcholanthrene increased cytochrome P-448 content, NADPH cytochrome-*c* reductase activity, 7-ethoxycoumarin *O*-deethylase activity and ethoxyresorufin *O*-deethylase but did not affect aminopyrine *N*-demethylase activity. This induction profile is qualitatively similar to that previously reported for the rat. There was no change in the ratio of urinary 6β-hydroxycortisol to 17-hydroxycorticosteroids. The pattern of response to 3-methylcholanthrene indicates that cortisol 6β-hydroxylase activity is not associated with induction of hepatic cytochrome P-448.

INTRODUCTION

The urinary excretion of 6β-hydroxycortisol (6β-OHC) has been shown to bear an empirical relationship to changes in mixed-function oxygenase activity produced by a range of enzyme inducers in man [1]. Further studies to elucidate the biochemical basis of this relationship required a suitable animal model. Although the guinea pig produces cortisol as its major corticoid and excretes 6β-OHC in urine we have found it to be unsuitable as an animal model [2]. Birchall *et al.* [3] found that phenobarbitone treatment produced large increases in 6β-OHC excretion in a New World monkey (*Cebus albifrons*) but did not measure hepatic enzyme activity. We [4] have shown previously that 6β-OHC is excreted in large amounts in the urine of marmoset monkeys and that phenobarbitone treatment produced large increases in 6β-OHC excretion and accompanying increases in cytochrome P-450 content, aminopyrine *N*-demethylase activity and ethoxycoumarin *O*-deethylase activity.

In this study, we have attempted to classify further the category of induction response with which 6β-hydroxylase activity is associated. In this paper we report the effects of 3-methylcholanthrene administration on hepatic microsomal mixed-function oxidase activity and protein profiles and urinary 6β-OHC excretion in the marmoset monkey.

MATERIALS AND METHODS

Chemicals. Tritiated 6β-hydroxycortisol (S.A. 52 Ci/mmol) was a gift from the Radiochemical Centre (Amersham, U.K.). Standard radioimmunoassay reagents were prepared as described previously [6]. Hydrazinobenzenesulphonic acid was obtained from Eastman Ltd., and purified by recrystallization [5]. Ketodase (β-glucuronidase) was purchased from Warner Lambert (U.K.) Ltd., 7-ethoxycoumarin and umbelliferone were obtained from Sigma Ltd., and ethoxyresorufin and resorufin from Pierce and Wariner Ltd. All other general reagents were obtained from B.D.H. All solvents were redistilled before use. Scintillant (NE 260) was obtained from Nuclear Enterprises.

Experimental animals. Eight male marmoset monkeys (bred at I.C.I. Pharmaceuticals Division, Alderley Park) weighing between 300 and 500 g were housed in individual metabolism cages. On day 0, control 24 hr urine samples were collected for each animal. On days 1, 2 and 3 four animals were injected intraperitoneally with 3-methylcholanthrene (25 mg/kg) in arachis oil and four animals were injected with arachis oil alone (2.5 ml/kg). Complete 24 hr urine collections were made on days 1, 2 and 3. After the final urine collection the animals were killed with carbon dioxide.

Methods. The preparation of liver microsomes, measurement of cytochrome P-450 content and model substrate metabolism and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) of microsomal proteins have been described [4]. Urinary 6β-hydroxycortisol excretion was determined by radioimmunoassay [4, 6] and 17-hydroxycorticosteroids were determined by a spectrophotometric assay after reaction with *p*-hydrazinobenzene sulphonic acid [4, 5].

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Table 1. The effect of 3-methylcholanthrene on liver weight, microsomal enzyme activity and microsomal cytochrome P-450 content in male marmosets

Treatment	Arachis oil	3-Methylcholanthrene
Body weight (g)	389 ± 38	342 ± 12
Liver weight (g/100 g body wt)	4.35 ± 0.24	4.14 ± 0.12
*Cytochrome P-448 (nmole/mg protein)	0.33 ± 0.02	0.44 ± 0.04*
NADPH Cytochrome-*c* reductase (nmole/min/mg protein)	126 ± 6	181 ± 10**
Aminopyrine *N*-demethylase (nmole/min/mg protein)	5.98 ± 0.50	5.77 ± 0.84
7-Ethoxycoumarin *O*-deethylase (nmole/min/mg protein)	0.45 ± 0.06	1.03 ± 0.13**
Ethoxyresorufin *O*-deethylase (nmole/min/mg protein)	0.036 ± 0.013	0.366 ± 0.069**

Values are given as means (n = 4) ± S.E.
Statistical significance from arachis oil treated controls using Student's t-test *P < 0.05, **P < 0.01.
* The λ_{max} in the reduced CO difference spectrum was 448.5 nm and therefore the induced cytochrome variant has been designated P-448.

RESULTS

The effect of 3-methylcholanthrene treatment on hepatic enzyme activity is shown in Table 1. 3-Methylcholanthrene treatment produced a large (10-fold) increase in ethoxyresorufin *O*-deethylase activity and smaller but significant increases in NADPH-cytochrome-*c* reductase activity (44 per cent), cytochrome P-450 (λ_{max} 448.5) content (33 per cent), and 7-ethoxycoumarin *O*-deethylase activity (129 per cent). There was no significant change in aminopyrine *N*-demethylase activity.

The effect of 3-methylcholanthrene treatment on urinary 6β-OHC, 17-hydroxycorticosteroids (17-OHCS) and the calculated ratio 6β-OHC/17-OHCS is shown in Fig. 1 and the corresponding data for arachis oil controls in Fig. 2. Both 3-methylcholanthrene and arachis oil produced significant increases in steroid output on days 1 and 2 compared to day 0. However, in neither case was there a change in the calculated ratio which remained constant throughout the experiment. There was no significant difference in either 6β-OHC or 17-hydroxycorticosteroid excretion between the two treatment groups on any of the days.

After 3-methylcholanthrene treatment, SDS PAGE indicated that only one protein band (mol. wt = 56,000) which was just visible in control microsomes was induced (Fig. 3). This pattern differed from that observed in liver microsomes from phenobarbitone treated marmosets where 3 protein bands (mol. wt. = 49,000, 52,000 and 55,000) were inten-

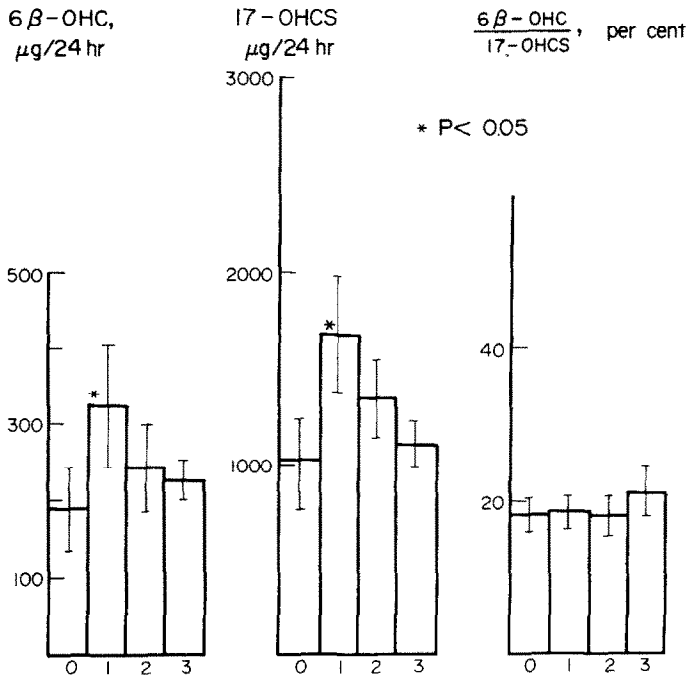

Fig. 1. The 24 hr urinary excretion of 6β-hydroxycortisol (6β-OHC), 17-hydroxycorticosteroids (17-OHCS) and the calculated ratio 6β-hydroxycortisol:17-hydroxycorticosteroids (6β-OHC/17-OHCS) in marmoset monkeys before (day 0) and during (days 1, 2 and 3) treatment with 3-methylcholanthrene 25 mg/kg. Results are means (n = 4) ± S.E. Statistical significance from control day using Student's t-test. *P < 0.05.

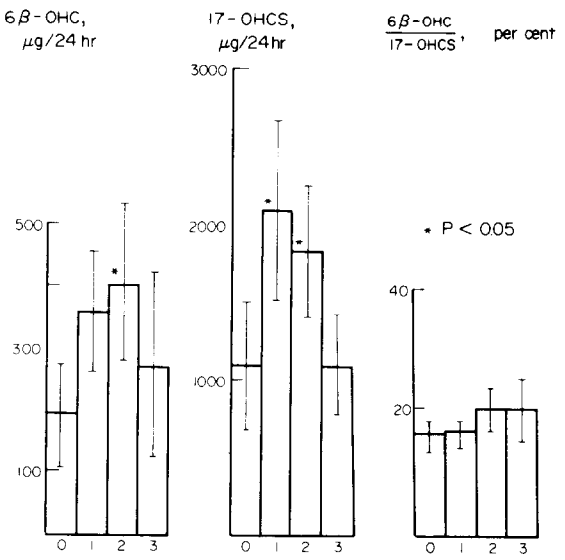

Fig. 2. The 24 hr urinary excretion of 6β-hydroxycortisol (6β-OHC), 17-hydroxycorticosteroids (17-OHCS) and the calculated ratio 6β-hydroxycortisol:17-hydroxycorticosteroids (6β-OHC/17-OHCS) in marmoset monkeys before (day 0) and during (days 1, 2 and 3) treatment with arachis oil. Results are means (n = 4) ± S.E. Statistical significance from control day using Student's t-test. *P < 0.05.

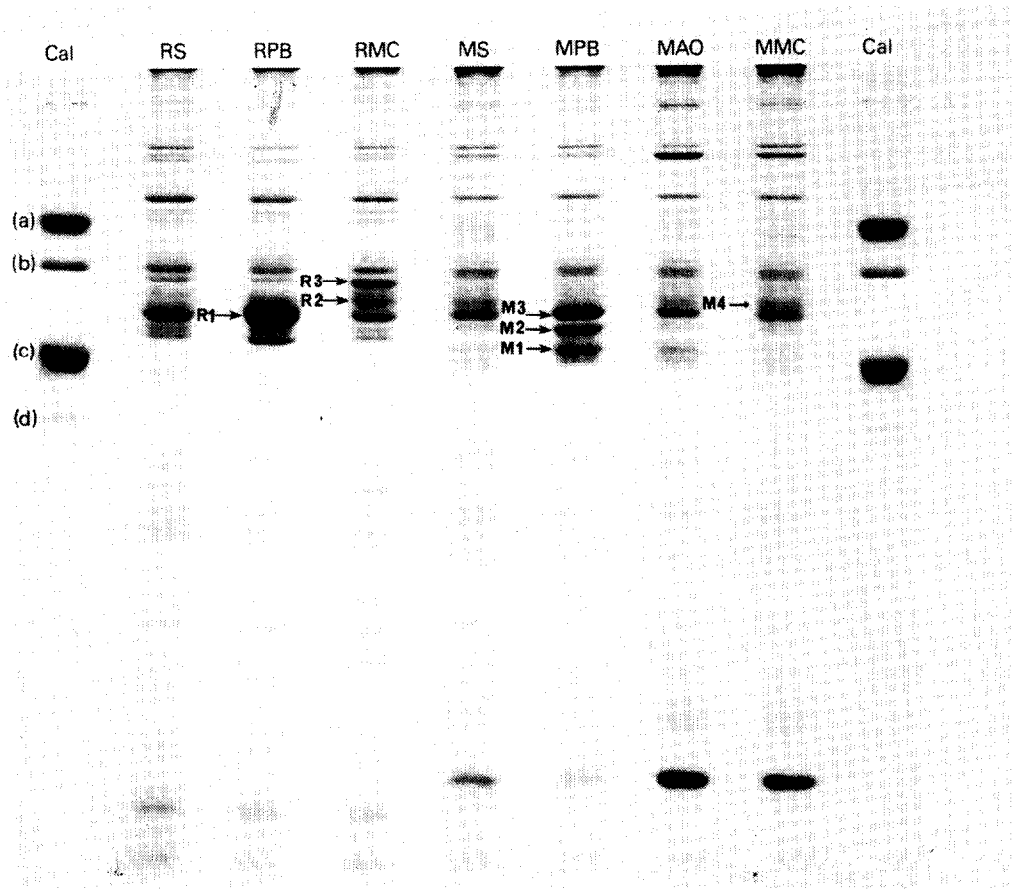

Fig. 3. Electrophoretogram of liver microsomes from marmoset treated with 3-methylcholanthrene. The induced protein bands are labelled with arrows. Abbreviations: Cal: Calibration proteins (a) bovine serum albumin MW 68,000 (b) catalase MW 60,000 (c) hen egg albumin MW 45,000 (d) aldolase MW 39,500. RS: rat saline control; RPB: rat phenobarbitone, band RI–MW 53,000; RMC: rat 3-methylcholanthrene band R2–MW 56,000 and band R3–MW 58,000. MS: marmoset saline control; MPB marmoset phenobarbitone, band M1–MW 49,000, band M2–MW 52,000 and band M3–MW 54,000; MAO: marmoset arachis oil control; MMC: marmoset 3-methylcholanthrene band M4–MW 56,000.

sified and from 3-methylcholanthrene treated rats where 2 bands (mol. wt. = 58,000 major and 56,000 minor) were increased.

DISCUSSION

Previous work [4] indicated that measurement of urinary 6β-OHC, when expressed as a percentage of total 17-hydroxycorticosteroids, is a useful index of induction of microsomal mixed-function oxidases by phenobarbitone in the marmoset monkey. Use of the ratio of 6β-OHC/17-OHCS rather than 6β-OHC excretion alone is favoured because of variations in cortisol production. Phenobarbitone produced increases in the ratio of 6β-OHC/17-OHCS with concomitant increases in cytochrome P-450 content, and those enzyme activities associated with phenobarbitone (P-450) induction in the rat [7]. Thus, there were significant increases in aminopyrine *N*-demethylase and ethoxycoumarin *O*-deethylase activities but no change in ethoxyresorufin *O*-deethylase activity with phenobarbitone. In the present study we found that administration of 3-methylcholanthrene had no effect on the ratio of 6β-OHC/17-OHCS in the marmoset monkey, although significant increases in hepatic enzyme activity were observed. The pattern of induction of model substrates was as found on 3-methylcholanthrene treatment of the rat [7]. There was a 10-fold increase in the rate of metabolism of ethoxyresorufin, a smaller increase in ethoxycoumarin *O*-deethylase activity (129 per cent) and no change in aminopyrine *N*-demethylase activity.

The lack of effect of 3-methylcholanthrene on urinary 6β-OHC/17-OHCS excretion suggests that cortisol 6β-hydroxylase activity is not associated with increases in cytochrome P-448 activity. Conney *et al.* [8] similarly found that treatment of immature male rats with phenobarbitone stimulated the 6β-hydroxylation of testosterone by liver microsomes *in vitro*, but that treatment with 3-methylcholanthrene had no effect on 6β-hydroxylase activity.

It is concluded that cortisol 6β-hydroxylation activity is associated with increases in cytochrome P-450 but not cytochrome P-448.

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