Pergamon

0960-0760(94)00119-7

J. Steroid Biochem. Molec. Biol. Vol. 51, No. 3/4, pp. 219–225, 1994

0960-0760/94 $7.00 + 0.00

Distinct Forms of Hepatic Androgen 6β-Hydroxylase Induced in the Rat by Indole-3-carbinol and Pregnenolone Carbonitrile

Peter H. Jellinck,[1]* Anne-Marie Newcombe,[1] P. Gek Forkert[2] and Charles P. Martucci[3]

Departments of [1]Biochemistry and [2]Anatomy and Cell Biology, Queen's University, Kingston, Ontario K7L 3N6, Canada and [3]Strang-Cornell Cancer Research Laboratory, 510 East 73rd Street, NY 10021, U.S.A.

The ability of indole-3-carbinol (IC), an anticarcinogen present in cruciferous vegetables, to induce CYP1A1, CYP1A2, CYP2B1/2, CYP2E1 and CYP3A1/2 in female rat liver was determined by Western analysis using monoclonal antibodies and compared to effects produced by pregnenolone carbonitrile in animals of both sexes. The ontogeny of induction of these cytochrome *P*450 isozymes in response to oral administration of IC was also investigated. An inverse correlation was observed between the 6β-hydroxylation of androsterone (A) and the induction by IC of CYP3A1/2, the *P*450 isozyme responsible for the bulk of hepatic 6β-hydroxylation of 4-androstenedione (AD). The effect of inhibitors on the formation of 6β-OHA from A or AD was also determined and shown to differ from their action on the *P*450 isozymes involved in the formation of the 6β-hydroxylated derivatives of AD or lithocholic acid. The results indicate that the enzyme induced by IC is distinct from the CYP3A1/2 which catalyzes hydroxylations at position 6β, allylic in AD but not in the fully saturated ring system of A. The increased hepatic conversion of A to its biologically less active 6β-OHA metabolite after treatment of female rats with IC could possibly contribute to the anticarcinogenic action of indole carbinols. It is also proposed that the action of multiple inducers present in cruciferous and other vegetables might produce androgen metabolic profiles very different from those produced by individual components isolated from them.

J. Steroid Biochem. Molec. Biol., Vol. 51, No. 3/4, pp. 219–225, 1994

INTRODUCTION

Currently, there is much interest in identifying those dietary components that could explain the epidemiological observations which correlate a diet high in vegetables with a lower incidence of breast [1], prostate [2], and colon cancer [3]. Cruciferous vegetables (cabbage, broccoli, cauliflower, Brussels sprouts, etc.) contain several components which have been observed to inhibit tumor formation in rodents, e.g. sulforaphane [4] and IC [5]. Sulforaphane appears to act by the induction of phase 2 enzymes. The mechanism for breast tumor inhibition by IC is thought to be mediated by the induction of estrogen-2-hydroxylation which diverts estrogen metabolism away from the more chemically reactive estrogen 16α-hydroxylation pathway [6]. We have shown recently [7] that IC, and its main gastric conversion product diindoylmethane (DIM), are able to alter the metabolism of androgens as well as estrogens. It was found that IC and DIM were able to induce the hepatic hydroxylases which catalyze the conversion of androsterone to its 6β-hydroxylated metabolite (3α,6β-dihydroxy-5α-androstane-17-one) without significantly affecting the 6β-hydroxylation of AD. These observations suggest that IC and DIM can induce a *P*450 isozyme(s) which is able to catalyze 6β-hydroxylation of androgens, with preference for 3α hydroxyl and 5α reduced substrates.

Hydroxylation at the C-6 position is a minor reaction for estrogens [8], but it is an important metabolic pathway for androgens, [9] glucocorticoids, progestins [10] and bile acids [11] in man as well as the rat. The 6α-hydroxylation of androgens and progestins is very tissue specific with microsomes from rat brain and

*Correspondence to P. H. Jellinck.
Received 9 June 1994; accepted 13 July 1994.
Abbreviations: IC, indole-3-carbinol; A, 3α-hydroxy-5α-androstane-17-one; AD, 4-androstene-3,17-dione; PCN, pregnenolone-16α-carbonitrile; 4-MA, 17β-*N*,*N*-diethyl-carbamoyl-4-methyl-4-aza-5α-androst-1-ene-3-one; 6-MPA, 6-medroxyprogesterone acetate.

prostate being more effective in catalyzing the reaction than liver [12]. The preferred substrate in these tissues is the 3β hydroxy 5α reduced steroid, which differs from the 6β-hydroxylation of the 3α hydroxy 5α reduced androgen induced in the liver by IC.

The present work provides further evidence for the existence of separate liver cytochromes *P*450 able to form the 6β isomer of AD and A, and examines the ontogeny of the expression of the isozymes CYP1A1, 1A2, CYP2B1/2 and CYP3A1/2 in female rat liver after oral administration of IC. It also compares the abilities of IC and PCN to induce CYP2B1/2, CYP2E1 and CYP3A1/2 in adult male and female rat livers. In addition, the responses of the induced enzyme(s) to various inhibitors of 6β-hydroxylation of A have been examined and shown to differ from either AD or lithocholic acid 6β-hydroxylase.

MATERIALS AND METHODS

Chemicals and reagents

[4-^{14}C]Androstenedione (53.9 mCi/mmol) purchased from New England Nuclear (Boston, MA) was shown by chromatography and autoradiography to be free of radioactive impurities. IC, Metyrapone (2-methyl-1,2-di-3-pyridyl-1-propanol), tamoxifen (1-[*p*-dimethylaminoethoxyphenyl]-1,2-diphenyl-1-butene), and the reduced coenzymes were obtained from Sigma Chemical Co. (St Louis, MO), the steroid androgens were from Steraloids (Wilton, NH) PCN from The Upjohn Co. (Kalamazoo, MI) and lithocholic acid from ICN Biomedicals Inc. (Cleveland, OH). Monoclonal antibodies prepared against CYP1A1 and CYP1A2 (Mab 1-7-1), CYP2B1/2 (Mab 2-66-3), CYP2E1 (Mab 1-98-1) and CYP3A1 (Mab 2-13-1) were a gift from Drs H. V. Gelboin and S. S. Park (Laboratory of Molecular Endocrinology, National Cancer Institute, Bethesda, MD).

Treatment of animals

Mature female (150–175 g), male (325–350 g) or immature Sprague–Dawley rats of various ages (Charles River, St Constant, Quebec) were maintained under standard conditions of light (0600–2000 h) and temperature (21–22°C) on a diet of Purina laboratory chow (Ralston-Purina, St Louis, MO) and water *ad libitum*. The animals were given IC (100 mg/kg) in sesame oil by stomach tube or PCN (3 × 50 mg/kg in oil) by daily intraperitoneal injections. Control animals received sesame oil alone. The rats were killed 20 h after the last treatment by cervical dislocation after CO_2 anesthesia.

Preparation of liver microsomal fraction and incubation

A 10% (w/v) homogenate of the liver was prepared in 0.25 M sucrose using a Potter–Elvehjem homogenizer with a Teflon pestle. The homogenate was centrifuged at 8000 *g* for 15 min and a microsomal fraction was obtained from the supernatant by centrifuging at 105,000 *g* for 1 h, washing with 0.5 ml of 0.25 M sucrose, and resuspending the pellet in sucrose. The microsomes derived from 200 mg of original tissue/ml could be stored at −70°C for several months without significant loss of activity. Protein was determined by the method of Lowry *et al.* [13]. The ^{14}C-labeled steroids (4.4 μM; ca. 1.2×10^5 dpm) were incubated with constant shaking at 37°C with the resuspended microsomes from 50 mg liver (approx. 0.8 mg protein) and NADPH (0.3 mM) in 0.1 M potassium phosphate (pH 7.4), in a total volume of 4 ml. Inhibitors were added in ethanol (10–25 μl) which was also present in the control tubes. The solution was then extracted three times with equal volumes of diethyl ether which was dried over anhydrous Na_2SO_4 and evaporated to dryness under N_2 at 25°C.

Separation and identification of metabolites

The residue was dissolved in ethanol (0.1–0.2 ml) and separated by TLC on silica gel using chloroform–ethyl acetate (1:2, v/v) twice, and visualized by autoradiography [7]. Areas of the TLC plate containing the metabolites were scraped directly into vials for determination of ^{14}C-radioactivity by scintillation counting. Non-radioactive androgen metabolites were visualized by their fluorescence after separation by TLC on silica gel plates containing a fluorescent indicator (precoated plates on aluminum backing, E. Merck, F-254) or else by spraying with Folin-Ciocalteu reagent diluted with water (1:2, v/v) or with H_2SO_4 in ethanol (1% v/v) and heating on a hot plate. Additional evidence of identity was provided by chromatography with standards in other TLC systems (chloroform–ethyl acetate–ethanol, 11:7:1 or chloroform–ethanol, 19:1, v/v). The main induced steroid metabolite of AD after treatment with IC has been shown previously by GC-MS to be 6β-OHA [7].

Nature of water-soluble products

To measure irreversible binding of the ^{14}C-labeled metabolites of AD and estradiol to protein and liver microsomes, 10 mg of bovine serum albumin (BSA) was added to 2 ml of the extracted aqueous medium to increase the yield of precipitate followed by 0.5 ml of HCl (IM) and 2 ml of 1.5 M trichloroacetic acid (TCA). The mixture was stirred by vortex and allowed to stand for 2–3 h at 25°C. It was then spun at 1000 *g* for 15 min and the precipitate washed with TCA (1 ml) followed by ethanol (2 × 2 ml) by repeated stirring and sedimentation at 1000 *g*. The residue was dissolved in 1 ml of NCS tissue solubilizer (Amersham) and 0.1 ml of this solution was counted.

Immunoblotting

This was carried out as described previously [14]. Liver microsomes (2–20 μg protein) were kept for

5 min at 95°C in the loading buffer [0.25 M Tris–HCl (pH 6.8), 10% (v/v) glycerol, 5% (v/v) β-mercaptoethanol, 2% (w/v) sodium dodecyl sulfate (SDS) and 0.001% (w/v) bromophenol blue] and electrophoresed on standard 9 × 14 cm 10% polyacrylamide gels [36] at 35 V for 18 h. The gel was transblotted for 4 h at 35 V in 22 mM Tris base–190 mM glycine–20% (v/v) methanol (pH 8.2) onto Hybond-ECL nitrocellulose, pore size: 0.45 μm (Amersham).

Non-specific binding to the blots was prevented by preincubation in 5% (w/v) skim milk powder–Tris saline [20 mM Tris–HCl (pH 7.6)/137 mM NaCl] containing 5% (v/v) Tween-20 (TBS-T) for 1 h at 22°C followed by washing (3 ×) with TBS-T. The blots were then incubated for 18 h with the diluted primary antibody in TBS containing 2.5% (w/v) skim milk powder and washed again (3 ×) with TBS-T before addition of the secondary antibody (sheep antimouse Ig) linked to horseradish peroxidase (Amersham) in TBS containing 2.5% (w/v) skim milk powder. They were incubated for 1 h and washed (3 ×) with TBS-T, and the protein–antibody complex was visualized using an enhanced chemiluminescence detection kit (Amersham) based on the oxidation of luminal by the peroxidase.

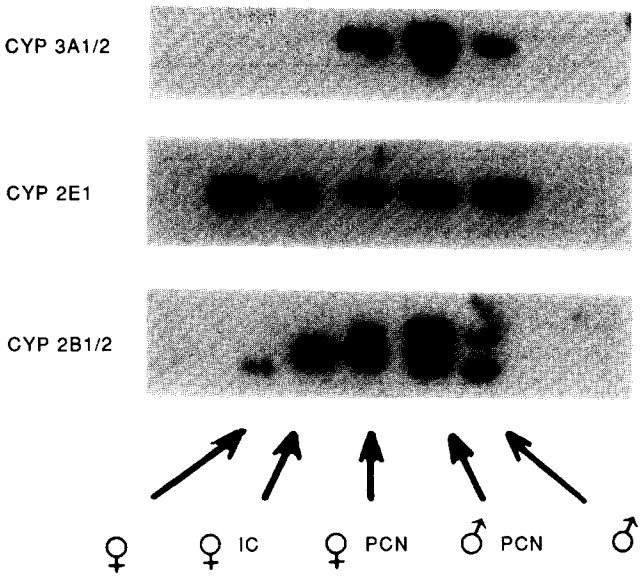

Fig. 1. Western blots of cytochrome *P*450 isozymes. Microsomal proteins from control and treated rats were separated by SDS-polyacrylamide gel electrophoresis and then subjected to Western blot analysis using monoclonal antibodies as described in Materials and Methods. Hepatic microsomal fractions are as follows: lane 1, control female (15.3 μg protein); lane 2, IC-treated female (20 mg, oral; 15.4 μg protein); lane 3, PCN-treated female (11 mg, i.p. ×3; 19.5 μg protein); lane 4, PCN-treated male (15 mg, i.p. ×3; 20.5 μg protein); lane 5 male control (17.7 μg protein).

RESULTS

Immunoblotting

The ability of IC to induce different cytochromes *P*450 in male and female rat livers was examined by Western blot analysis using monoclonal antibodies and compared to the effects produced by PCN (Fig. 1). CYP3A1, a steroid-inducible testosterone 6β-hydroxylase which also has a male-specific isoform (CYP3A2) was shown, by using a monoclonal antibody (Mab 2-13-1), to be induced by PCN in both sexes but not significantly by IC (immunoblot for males treated with IC is not shown). The level of hepatic CYP2E1 (tested with Mab 1-98-1) was not affected significantly by treatment with either PCN or IC but both these compounds were good inducers of CYP2B1/2 in male and female rats as demonstrated by using Mab 2-66-3. The response of female rats of various ages to the hepatic induction of CYP1A1 and 1A2, CYP2B1/2 and

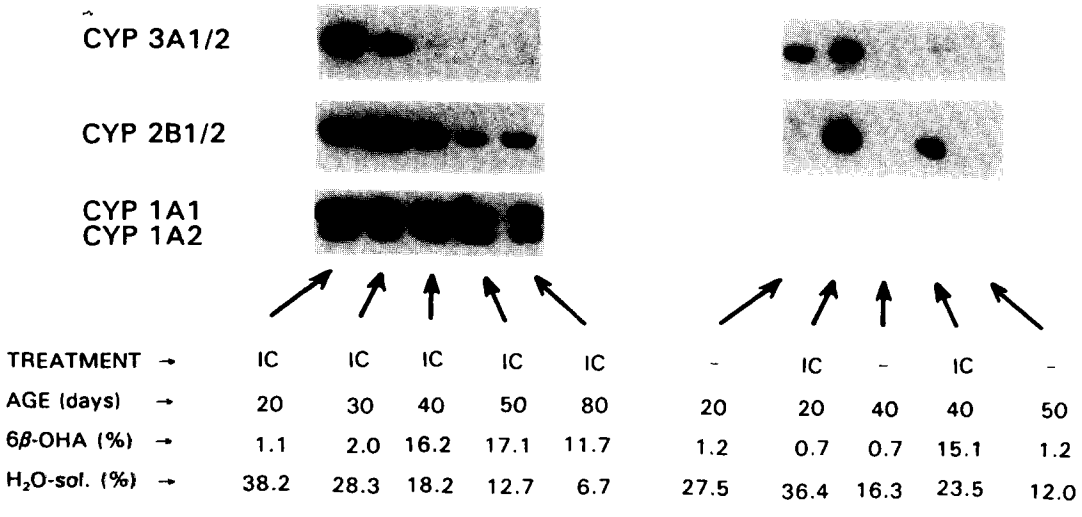

Fig. 2. Ontogeny of induction of CYP1A1, CYP1A2, CYP2B1/2 and CYP3A1/2 and correlation with 6β-hydroxylation of A and AD. Cytochrome *P*450 isozymes from female rats treated with IC (100 mg/kg) at different ages were detected by Western analysis as described in Fig. 1 and the conversion of AD to 6β-OHA by the same microsomal preparations was also measured as described in Materials and Methods.

Table 1. Comparative binding of ^{14}C-labeled estradiol (E_2) and AD to protein after incubation with liver microsomes from male or female rats treated with IC or PCN

Treatment	Substrate	Water-soluble products (%)	Water-soluble products		
			Supernatant (%)	Ethanol wash (%)	Protein-bound (%)
Male	AD	19.8 (42.6)	78.2 (85.3)	14.0 (4.7)	2.7 (2.5)
	E_2	49.6 ± 2.9 (76.4)	44.6 (91.4)	26.4 (6.0)	26.4 (1.2)
Male PCN	AD	51.5 ± 3.7 (65.2)	87.2 ± 2.6 (86.1)	4.9 ± 0.5 (3.4)	2.1 ± 0.4 (0.9)
	E_2	52.5 (75.4)	41.5 (84.3)	30.6 (9.8)	20.0 (3.6)
Female	AD	11.0 (14.3)	78.0 (75.4)	17.7 (8.4)	3.9 (3.9)
	E_2	21.5 (38.4)	34.0 (78.5)	41.9 (8.1)	29.0 (0.7)
Female IC	AD	16.4 ± 0.7 (17.4)	73.7 ± 1.9 (83.2)	15.7 ± 1.2 (10.4)	4.1 ± 1.0 (3.7)
	E_2	44.5 (62.5)	22.2 (82.6)	55.8 (6.7)	22.2 (0.8)

Microsomes from the liver (50 mg) of rats treated as indicated were incubated with [4-^{14}C]AD or E_2 (4.4 μM) and NADPH (0.3 mM) for 30 min before extraction with ether. BSA was then added to the water-soluble fraction and, after precipitation with TCA, the pellet was washed twice with ethanol and the residue dissolved in NCS tissue solubilizer for determination of ^{14}C-radioactivity. Other conditions are as described in Materials and Methods. Values are means of 2 experiments or means ± SEM from 4 experiments; those in parentheses are from incubations with added GSH (5 mM).

CYP3A1/2 after oral administration of IC is shown in Fig. 2. CYP1A1 and 1A2 could be induced at all ages between 20 and 80 days while CYP2B1/2 showed maximum response at 30 days of age followed by a gradual decrease. CYP1A2 was present in trace amounts in untreated animals (not shown) while CYP3A1/2 was shown to occur constitutively in 20-day-old rats but to be absent at 40 or 50 days and no longer inducible by IC in these older animals. Others have found previously [15, 16] that the 6β-hydroxylation of androstenedione and testosterone by female rat liver microsomes reaches a maximum around 20–30 days of age. The extent of 6β-hydroxylation of A did not coincide with the amounts of CYP3A1/2 present which paralleled the 6β-hydroxylation of AD and the formation of water-soluble products not extractable by ether.

Covalent binding

Some of the radioactive products present in the water-soluble fraction co-precipitated with protein but most of them could be removed by washing the pellet with ethanol unlike when [^{14}C]estradiol was used as substrate (Table 1). Therefore, the metabolites are not bound covalently and might represent highly polar ether-insoluble derivatives. However, the addition of glutathione (GSH) to the incubation mixture generally increased the yield of water-soluble products and in the case of [^{14}C]estradiol also decreased the percentage of ^{14}C-radioactivity bound to protein. Amines such as cadaverine, putrescine and spermine had no effect on any of the reactions which were completely abolished by previous boiling of the liver microsomes.

Table 2. Effect of inhibitors on the conversion of ^{14}C-labeled AD or A to 6β-OHA by liver microsomes from IC-treated rats

Additions	Conc. (μM)	Conversion to 6β-OHA (%)			
		AD	Inhibition (%)	A	Inhibition (%)
Control	—	18.2 ± 1.1	—	26.6 ± 1.4	—
Metyrapone	1	10.9	40	17.3	35
	10	3.1 ± 0.5	83	3.0 ± 0.7	88
Tamoxifen	10	10.9 ± 0.8	40	23.2 ± 1.9	13
	25	10.2 ± 3.2	44	24.0 ± 0.3	10
Lithocholic acid	10	16.1 ± 0.9	12	28.5 ± 2.0	0
	25	14.8 ± 1.5	19	27.3 ± 7.6	0
4-MA	1	3.4 ± 0.8	81	27.6 ± 4.8	0
	10	1.4 ± 0.2	92	23.7 ± 3.9	11
6-MPA	10	6.7 ± 0.5	63	24.1 ± 3.0	9
	25	4.5 ± 1.2	75	23.3 ± 4.8	12

Microsomes from IC-treated female rats were incubated with [4-^{14}C]AD or [4-^{14}C]A (4.4 μM) and NADPH (0.3 mM) for 30 min in the presence or absence of inhibitors before extraction with ether and separation of products by TLC ($CHCl_3$–ethyl acetate 1:2, v/v, × 2). Other conditions as described in Materials and Methods. Values are means ± SEM from 3 experiments.

Comparative effect of inhibitors

The effect of various inhibitors on the formation of 6β-OHA from A or AD is shown in Table 2. Metyrapone was found to be a good inhibitor as shown previously [17] for the 6β-hydroxylation of AD but not lithocholic acid while tamoxifen differed in producing only weak inhibition of 6β-OHA formation. The addition of lithocholic acid had no effect showing that it was not competing for the enzyme induced by IC. As expected, a 5α-reductase inhibitor 4-MA [18] prevented the formation of 6β-OHA from AD but not A while 6-MPA, a 3α-hydroxysteroid dehydrogenase inhibitor [19], showed a similar but less pronounced effect on the conversion of AD to 6β-OHA. However, it did not inhibit the 6β-hydroxlation of A which is already hydroxylated at C-3.

DISCUSSION

In recent studies, we have shown that IC as well as its dimer when given orally, induce CYP1A1, 1A2, 2B1/2 and possibly other cytochrome *P*450 isozymes in rat liver [7]. The indole carbinols resembled isosafrole more closely than 3-methylcholanthrene in their induction specificities and they increased the conversion of estradiol to its catechol derivatives [6] as well as altering androgen metabolism. With the latter group of steroids, an increase in the formation of 6β-OHA was observed [7] without a concomitant increase in 6β-hydroxylation of the parent compound, 4-androstenedione.

Two closely homologous (90% with respect to amino acids) cytochrome *P*450 isozymes are known to catalyze the 6β-hydroxylation of androgens [16, 20, 21]. CYP3A1 is a PCN-inducible testosterone 6β-hydroxylase and appears not to be present constitutively in male or female rat hepatic tissues at any age, while CYP3A2 is a male-specific testosterone 6β-hydroxylase. By means of Western analysis using Mab 2-13-1 against CYP3A1, we showed the absence of CYP3A1 in adult female rat liver and its inducibility by PCN but not by IC. However, CYP3A2, which is known to be present in rats up to just after 30 days of age appeared to cross-react with Mab 2-13-1 and to be inducible by IC in these younger animals. CYP3A2 is known to be suppressed in adult females which develop a more continuous growth hormone secretory profile than males [15, 22, 23]. Thus, the indole carbinol could be inducing either or both isozymes particularly since the antibody gave a positive immunoreaction with liver microsomes from untreated adult male rats known to contain CYP3A2; it is for this reason they are designated CYP3A1/2 in this paper. The ability of monoclonal antibodies to cross-react with different members of the CYP3A subfamily has been described by others [24–26]. The CYP3A1/2 levels were paralleled by the extent of 6β-hydroxylation of AD but not of A. The 6β-hydroxylation of A, the fully reduced metabolite of AD, occurred only in IC-induced mature female rats when CYP3A1/2 was shown to be no longer present. A similar developmental pattern was observed with CYP2B1/2 [16, 20] although its decrease was delayed by about a week and, unlike CYP3A1/2, it could be induced by IC in adult female rat liver. CYP2B1/2 levels were increased by PCN or IC treatment in both sexes but neither this steroid nor IC had any significant effect on the expression of ethanol-inducible CYP2E1 [20, 27]. However, these isozymes are not associated with the 6β-hydroxylation of androgens.

Thus, IC induces a cytochrome *P*450 capable of catalyzing hydroxylation at the β face of A at C-6 which differs from the subfamily CYP3A1/2 responsible for the bulk of microsomal 6β-hydroxylation of AD in rat liver [16]. It is unlikely to be the form which catalyzes the 6β-hydroxylation of bile acids such as lithocholic acid [17] because it reacted differently to hydroxylase inhibitors such as metyrapone and tamoxifen and was not inhibited by lithocholic acid which would compete for the enzyme. In addition, androsterone, the substrate for the induced enzyme [7] is essentially planar while ring A of lithocholic acid, because of the 5β configuration, is bent at an almost right angle relative to the plane of the molecule [17]. CYP1A1, CYP1A2, CYP2B1/2 and CYP2E1 can also be excluded because their levels at different ages did not correspond to 6β-hydroxylation of A or response to IC administration.

It is interesting that humans produce significant amounts of 6α and 6β-hydroxylated bile acids during neonatal life, and again following the development of biliary obstruction [28]. Most of these 6α-hydroxylated bile acids appear to be excreted in the urine as C-6 conjugates [29]. Which endogenous compounds are controlling 6-hydroxylation of bile acids in humans is unknown; sex steroid hormones are known to control 6-hydroxylation in rats [30].

The fact that 6β-hydroxylation of A and AD is catalyzed by distinct enzymes, only one of which is induced by IC, has interesting biochemical implications. It has been pointed out that from the substrate structure–activity relationship for the CYP3A family of isozymes, hydroxylation takes place on a benzyl or allyl carbon atom [31]. The 6β-position is allylic in AD but not in the fully saturated ring system of A, making the former steroid molecule a much more suitable substrate for attack by CYP3A1/2. In this context, IC would be increasing the conversion of A to its inert 6β-OHA metabolite, possibly at the expense of 6β-OHAD capable of forming highly polar products. This is supported by the observed increase in polar water-soluble metabolites under conditions where 6β-OHAD rather than 6β-OHA is formed. It could also reflect increased imine formation by AD with lipophilic amines associated with liver microsomes which has

been shown to yield the 6β-hydroxy derivative by non-enzymatic hydrolysis [32]. However, the possibility of such a reaction and also peroxidation of C-6 of the steroid [33] can be eliminated because polyamines did not influence the yield of the 6β-hydroxylated androgens and boiling the microsomes completely abolished their formation.

Thus, the alterations in metabolism of AD produced after the ingestion of IC could affect the potential of this steroid to exert deleterious effects by increasing the hepatic conversion of AD to its inert 6β-OHA derivative. This would result in the decreased availability of AD to be aromatized to estrone in target tissues with subsequent reduced formation of 16α-hydroxyestrone which has been shown to function as an initiator and promoter of mammary cell transformation [34]. In humans, androgens are known to play a role in the development of prostate cancer and benign prostatic hypertrophy but the effect of androgen metabolism in these diseases is poorly understood [35]. Recently 5α-reductase inhibitors have come into clinical use for the treatment of benign prostatic hypertrophy; however, the efficacy of these drugs is limited and side effects can occur [36]. Clinical trials are in progress to determine if these agents are able to reduce the incidence of prostate cancer and it is clear that these agents can reduce the serum level of dihydrotestosterone, as well as the dihydrotestosterone content of the prostate gland. If the effect of IC on the induction of a 6β-hydroxylase (which requires a 5α reduced substrate) as shown here for the rat, also occurs in humans, then a pathway for the removal of androgens may be blocked following treatment with 5α-reductase inhibitors. This might explain the observation that testosterone levels are elevated in men undergoing 5α-reductase inhibitor therapy [36].

Epidemiological studies indicate that the consumption of a high vegetable diet is associated with reduced prostate cancer [2]. The mechanism for this dietary effect is unknown. It is possible that the apparent antitumor effect of a high vegetable diet could be mediated by the combined action of dietary inducers of phase 1 enzymes (e.g. IC), and other dietary inducers of phase 2 enzymes (e.g. sulforaphane) which are capable of conjugating the hydroxylated steroids produced by the phase 1 enzymes. The net effect in human males might be to protect the prostate by decreasing the levels of active androgens reaching this organ. Thus a possible synergistic action of the multiple inducers present in cruciferous and other vegetables could produce androgen metabolic profiles very different from those produced by individual components isolated from the vegetables. Recently, it has been shown [37, 38] that dietary IC can induce both phase 1 and 2 enzymes in the livers of mature male Fischer rats and that this multiple effect is probably brought about by the various metabolites formed from IC in the gastrointestinal tract.

Studies to characterize the nature of the water-soluble androgen metabolites produced in the rat following treatment with IC should provide some information about the type of reactions involved, and deserves further investigation. In addition, the significance of these *in vitro* changes will require testing *in vivo* by monitoring changes in circulating and urinary excretion products after the administration of IC.

Acknowledgement—This work was supported by grants from the Medical Research Council of Canada (P.H.J. and P.G.F.).

REFERENCES

1. Kelsey J. L. and Gammon M. D.: The epidemiology of breast cancer CA-A. *Cancer J. Clin.* **41** (1991) 147.
2. Bosland M. C.: The etiopathogenesis of prostate cancer with special reference to environmental factors. *Adv. Cancer Res.* **51** (1988) 1–106.
3. Howe G. R., Benito E., Castelleto R., Cornée J., Estéve J., Gallagher R. P., Iscovich J. M., Deng-ao J., Kaaks R., Kune G. A., L'Abbé K. A., Lee H. P., Lee M., Miller A. B., Peters R. K., Potter J. D., Riboli E., Slattery M. L., Trichopoulos D., Tayns A., Tzonou A., Whittemore A. S., Wu-Williams A. H. and Shu Z.: Dietary intake of fibre and decreased risk of cancers of the colon and rectum from the combined analysis of 13 case-control studies. *J. Natn. Cancer Inst.* **84** (1992) 1887–1886.
4. Zhang Y., Kensler T. W., Cho C.-G., Posner G. H. and Talalay P.: Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. *Proc. Natn. Acad. Sci. U.S.A.* **91** (1994) 3147–3150.
5. Bradlow H. L., Michnovicz J. J., Telang N. T. and Osborne M. P.: Effect of dietary indole-3-carbinol on estradiol metabolism and spontaneous mammary tumours in mice. *Carcinogenesis* **12** (1991) 1571–1574.
6. Jellinck P. H., Michnovicz J. J. and Bradlow H. L.: Influence of indole-3-carbinol on the hepatic microsomal formation of catechol estrogens. *Steroids* **56** (1991) 446–450.
7. Jellinck P. H., Makin H. L. J., Sepkovic D. W. and Bradlow H. L.: Influence of indole carbinols and growth hormone on the metabolism of 4-androstenedione by rat liver microsomes. *J. Steroid Biochem. Molec. Biol.* **46** (1993) 791–798.
8. Martucci C. P. and Fishman J.: *P*450 enzymes of estrogen metabolism. *Pharmac. Ther.* **57** (1993) 237–257.
9. Schenkman J. B.: Steroid metabolism by constitutive cytochromes *P*450. *J. Steroid Biochem. Molec. Biol.* **43** (1992) 1023–1030.
10. Hall P.: Cytochromes *P*450 and the regulation of steroid synthesis. *Steroids* **48** (1986) 131–196.
11. Zimniak P., Holsztynska E. J., Lester R., Waxman D. and Radominska A.: Detoxification of lithocholic acid. Elucidation of the pathways of oxidative metabolism in rat liver microsomes. *J. Lipid Res.* **30** (1989) 907–918.
12. Strömstalt M., Warner M., Banner C. D., MacDonald P. C. and Gustafsson J.-Å.: Role of brain cytochrome *P*450 in regulation of the level of anaesthetic steroids in brain. *Molec. Pharmac.* **44** (1993) 1077–1083.
13. Lowry O. H., Rosebrough N. J. and Randall R. J.: Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193** (1951) 265–275.
14. Jellinck P. H., Forkert P. G., Riddick D. S., Okey A. B., Michnovicz J. J. and Bradlow H. L.: Ah receptor binding properties of indole carbinols and induction of hepatic estradiol hydroxylation. *Biochem. Pharmac.* **45** (1993) 1129–1136.
15. Yamazoe Y., Shimada M., Murayama N., Nagata K. and Kato R.: Hormonal regulation of hepatic cytochrome *P*-450. In *Xenobiotic Metabolism and Disposition* (Edited by R. Kato, R. W. Estabrook and M. N. Cayen). Taylor and Francis, New York (1989) pp. 37–43.
16. Waxman D. J., Dannan G. A. and Guengerich F. P.: Regulation of rat hepatic cytochrome *P*-450: Age dependent expression,

hormonal imprinting, and xenobiotic inducibility of sex-specific isozymes. *Biochemistry* **24** (1985) 4409–4417.
17. Zimniak P., Holsztynska E. J., Radsminska A., Iscan M., Lester R. and Waxman D. J.: Distinct forms of cytochrome *P*450 are responsible for 6β-hydroxylation of bile acids and of neutral steroids. *Biochem. J.* **275** (1991) 105–111.
18. Liang T. and Heiss C. E.: Inhibition of 5α-reductase, receptor binding, and nuclear uptake of androgens in the prostate by a 4-methyl-4-aza-steroid. *J. Biol. Chem.* **256** (1981) 7998–8005.
19. Penning T. M., Sharp R. B. and Krieger N. R.: Purification and properties of 3α-hydroxysteroid dehydrogenase from rat brain cytosol. *J. Biol. Chem.* **260** (1985) 15,266–15,272.
20. Waxman D. J.: Interaction of hepatic cytochromes *P*450 with steroid hormones. Regioselectivity and stereospecificity of steroid metabolism and hormonal regulation of rat *P*-450 enzyme expression. *Biochem. Pharmac.* **37** (1988) 71–84.
21. Namkung M. J., Yang H. L., Hulla J. E. and Juchau M. R.: On the substrate specificity of cytochrome *P*450IIIA1. *Molec. Pharmac.* **34** (1988) 628–637.
22. Waxman D. J., LeBlanc B. A., Morrissey J. J., Staunton J. and Lapenson D. P.: Adult male specific and neonatally programmed rat hepatic *P*-450 forms RLM2 and 2a are not dependent on pulsatile plasma growth hormone for expression. *J. Biol. Chem.* **263** (1988) 11,396–11,406.
23. Yamazoe Y., Murayama N., Shimada M., Yamauchi K., Nagata K., Imaska S., Funae Y. and Kato R.: A sex-specific form of cytochrome *P*-450 catalyzing propoxycoumarin o-depropylation and its identity with testosterone 6β-hydroxylase in untreated rat livers. *J. Biochem.* **104** (1988) 785–790.
24. Gonzales F. J., Song B. J. and Hardwick J. P.: Pregnenolone 16α-carbonitrile-inducible P-450 gene family: gene conversion and differential regulation. *Molec. Cell. Biol.* **6** (1986) 2969–2976.
25. Halpert J. R.: Multiplicity of steroid-inducible cytochromes *P*-450 in rat liver microsomes. *Archs Biochem. Biophys.* **263** (1988) 59–68.
26. Cooper K. O., Reik L. M., Jayyosi A., Bandiera S., Kelley M., Ryan D. E., Daniel R., McCluskey S. A., Levin W. and Thomas P. E.: Regulation of two members of the steroid-inducible cytochrome *P*450 subfamily (3A) in rats. *Archs Biochem. Biophys.* **301** (1993) 345–354.
27. Ryan D. E., Koop D. R., Thomas P. E., Coon M. J. and Wayne L.: Evidence that isoniazid and ethanol induce the same microsomal cytochrome *P*-450 in rat liver, and isozyme homologous to rabbit liver cytochrome *P*-450 isozyme 3a. *Archs Biochem. Biophys.* **246** (1986) 633–644.
28. Strandvik B. and Wikström S.-A.: Tetrahydroxylated bile acids in healthy human newborns. *Eur. J. Clin. Invest.* **12** (1982) 301–305.
29. Sacquet E., Parquet M., Riottot M., Raizman A., Jarrige P., Huguet C. and Infante R.: Intestinal absorption, excretion and biotransformation of hyodeoxy choleic acid in man. *J. Lipid Res.* **24** (1983) 604–613.
30. Kern F., Eriksson H., Curstedt T. and Sjövall J.: Effect of ethynyl estradiol on biliary excretion of bile acids, phosphatidylcholines, and cholesterol in the bile fistula rat. *J. Lipid Res.* **18** (1977) 623–634.
31. Smith D. A. and Jones B. C.: Speculations on the substrate structure-activity relationship (SSAR) of cytochrome *P*450 enzymes. *Biochem. Pharmac.* **44** (1992) 2089–2098.
32. Eriksson C. G., Eneroth P. and Nordstrom L.: 6-oxygenation of 3-oxo4-ene-steroids in high yield after 3-imine formation. *J. Steroid Biochem.* **22** (1985) 649–655.
33. Eriksson C. G. and Eneroth P.: Peroxidation in position C-6 of progesterone-3-ethanolimine is increased by the presence of enzymes generating oxygen radicals. *Steroids* **55** (1990) 366–372.
34. Telang N. T., Suto A., Wong G. Y., Osborne M. P. and Bradlow H. L.: Induction by estrogen metabolite 16α-hydroxyestrone of genotoxic damage and aberrant proliferation in mouse mammary epithelium. *J. Natn. Cancer Inst.* **84** (1992) 634–638.
35. Bartsch W., Klein H., Schiemann U., Bauer H. W. and Voigt K. D.: Enzymes of androgen formation and degradation in the human prostate. *Ann. N.Y. Acad. Sci.* **595** (1990) 53–66.
36. Gormley G. J., Stoner E., Bruskewitz R. C., Imerate-McGinley J., Walsh P. C., McConnell J. D., Andriole G. L., Geller J., Bracken B. R., Tenover J. S., Vaughan E. D., Pappas F., Taylor A., Binkowitz B. and Ng J.: The effect of finasteride in men with benign prostatic hyperplasia. *New Engl. J. Med.* **327** (1992) 1185–1191.
37. Stresser D. M., Bailey G. S. and Williams D. E.: Indole-3-carbinol and β-naphthoflavone induction of aflatoxin B_1 metabolism and cytochromes *P*-450 associated with bioactivation and detoxication of aflatoxin B_1 in the rat. *Drug Metab. Dispos.* **22** (1994) 383–391.
38. Stresser D. M., Williams D. E., McLellan L. I., Harris T. M. and Bailey G. S.: Indole-3-carbinol induces a rat liver glutathione transferase subunit (Yc2) with high activity towards aflatoxin B_1 exo-epoxide. *Drug Metab. Dispos.* **22** (1994) 392–399.