Biochemical Pharmacology, Vol. 22, pp. 2875–2880. Pergamon Press, 1973. Printed in Great Britain.

THE EFFECT OF DIETARY PROTEIN AND FAT ON THE ACTIVITY OF ARYL HYDROCARBON HYDROXYLASE IN RAT LIVER, KIDNEY AND LUNG

ALAN J. PAINE and ANDRÉ E. M. MCLEAN

Department of Experimental Pathology, University College Hospital Medical School, University Street, London WC1E 6JJ, England

(*Received* 7 *February* 1973; *accepted* 9 *May* 1973)

Abstract—Feeding low protein diets reduces the activity of aryl hydrocarbon hydroxylase (3,4 benzo(α)pyrene hydroxylase) in rat liver and lung, but not in the kidney. In the kidney the level of aryl hydrocarbon hydroxylase activity is increased by feeding a diet containing fats. This increase in the kidney aryl hydrocarbon hydroxylase activity occurs when the fat content of the diet rises above 4 per cent and is maximal after feeding such diets for 7 days.

THE MICROSOMAL cytochrome P450 associated oxidative enzyme system, of which aryl hydrocarbon hydroxylase (AHH) is a component, is important in the metabolism of a variety of compounds including many drugs, hormones and carcinogens.

AHH from rat liver metabolises a variety of polycyclic hydrocarbons to polar derivatives; other tissues also have this activity especially the kidney, lung, skin and gastrointestinal tract.[1]

Changes in nutrition alter the level of the liver microsomal hydroxylating enzymes; the important dietary factors have been shown to be the protein content of the diet,[2,3] the unsaturated fat content and its state of oxidation,[4] the presence of non-nutrient inducing components such as Flavones.[5]

Because of the possible importance of microsomal hydroxylating enzymes in chemical carcinogenesis,[6,7] we have investigated the effect of diet on the activity of AHH in liver, kidney and lung. We hope in this way to provide further insights into the suggestion made by Doll,[8] and many others, that the environment and especially diet plays a major role in carcinogenesis.

MATERIALS AND METHODS

Rats. Male Wistar rats (A. Tuck & Son, Rayleigh, Essex, U.K.) weighing 110–140 g, were housed in cages with wire grid bottoms and so denied access to sawdust and faeces as far as possible.

Diets. Stock diets, M.R.C. 41B (9), (Oxoid Ltd., London S.E.1., U.K.) or semi-synthetic diets made as previously described,[4] were fed *ad lib.* up to the time of death.

Abbreviations: AHH aryl hydrocarbon hydroxylase; BP benzo(α)pyrene.

Tissue samples. Each rat was killed between 10.00 and 11.00 hr by exsanguination from the carotid artery under ether anaesthesia. The tissues were excised, rinsed in ice-cold isotonic saline, blotted dry and weighed.

Ten per cent liver homogenates (i.e. 1 g liver + 9 ml KCl) and 5 per cent kidney or lung homogenates were made in 0·15 M KCl using an Ultra-Turrax type TP 18/2 blender (Janke & Kunkel A.G., Staufen i.Br., Germany) for 8 sec.

Post mitochondrial fraction was prepared by pipetting 10 ml of the homogenate into a 50 ml tube and centrifuging for 10 min at 7000 *g* (max) in a refrigerated centrifuge.

Chemicals. DL-Isocitrate, isocitrate dehydrogenase (EC 1.1.1.42), bovine serum albumin, 3,4 benzo(*a*)pyrene, cytochrome *c* type III, and NADPH type 1 were obtained from Sigma Chemical Co. (London). NADP di sodium salt was obtained from Boehringer Corporation (London). All other chemicals were Analar grade and purchased from British Drug Houses. (Poole, Dorset). 3-hydroxy benzo(*a*)pyrene was a generous gift from Dr. H. V. Gelboin.

Assays in vitro. AHH activity was assayed, using 3,4 benzo(*a*)pyrene as the substrate by the method described by Nebert and Gelboin[10], with the following modification to the incubation mixture. The incubation mixture in a final volume of 1·0 ml contained an NADPH generating system composed of 3·3 μmoles DL isocitrate, 0·35 μmole NADP, 0·2 i.u. isocitric dehydrogenase. In addition bovine serum albumin (0·9 mg/ml) was included to solubilize 3,4 benzo(*a*)pyrene.[11]

To this incubation mixture was added 5 mg liver (0·6–1 mg protein) or 10 mg kidney or lung (1·3–1·8 mg and 0·7–1·2 mg protein respectively). After a 2-min pre-incubation at 37° the reaction was started by the addition of 200 nmoles of 3,4 benzo(*a*)pyrene in 20 μl acetone. The mixture was shaken at 37° in air for 10 min and the reaction was then stopped by the addition of 1·0 ml ice-cold acetone followed by 3 ml hexane (b.p. 67°–70°) and the mixture was shaken at 37° for 20 min. A 1 ml sample of the organic phase was extracted into 4 ml of 1 M NaOH for incubations with liver homogenates; for incubations with kidney and lung homogenates 2 ml of the organic phase was extracted into 0·5 ml 1 M NaOH.

3-Hydroxy benzo(*a*)pyrene was used as the primary standard and quinine sulphate as a working standard in the spectrophotofluorimetric assay of the metabolites present in the extracts of the incubation mixture.

The results are expressed as pmoles of 3-hydroxy benzo(*a*)pyrene formed.

Enzyme activities were determined in duplicate and compared to a blank to which 1 ml of acetone had been added prior to incubation.

NADPH: cytochrome *c* reductase (EC 1.6.2.3.) activity was determined by the method of Phillips and Langdon.[12]

Cytochrome P-450 was measured in homogenates by the method of Omura and Sato[13] as modified by Griem[14] on a Unicam SP800 spectrophotometer in conjunction with an external recorder. For this determination the homogenate and Post Mitochondrial Supernatant were diluted with sodium phosphate buffer (final concn 50 mM phosphate pH 7·4) such that 1 ml contained 20 mg liver.

Protein was determined by the method of Lowry *et al.*[15] using crystalline bovine serum albumin as a reference standard.

Statistics. The student's *t*-test was used as a test of significance between sample means.

RESULTS

NADPH cytochrome *c* reductase activity was measured in homogenates and post-mitochondrial supernatant fraction of liver, kidney and lung; cytochrome P-450 content was also measured in liver fractions.

When these microsomal marker enzymes were measured in both homogenate and post-mitochondrial fraction it was found that 20–50 per cent of the microsomes were precipitated at 70,000 *g* min in KCl. An equivalent loss of AHH activity was also found, while homogenate activities were reproducible, and proportional to time and amount of tissue.[11] From these considerations we decided that homogenates were the most suitable preparations for measuring changes in AHH activity of tissues from animals in varying nutritional states.

Table 1 shows the effect of feeding different diets to rats upon the AHH activity in liver, kidney and lung. The level of liver and lung AHH in rats fed a no protein (high carbohydrate) or a low protein diet (3% casein) is significantly lower than that found in rats fed a high protein (20% casein) or the stock diet ($P < 0{\cdot}05$ in these cases).

TABLE 1. THE EFFECT OF DIET UPON AHH ACTIVITY IN LIVER, KIDNEY AND LUNG

Diet	AHH activity pmoles 3-hydroxy b.p. formed/30 min/mg protein		
	Liver	Kidney	Lung
No-fat no-protein (65% cornflour, 35% vitamin supplement)	1163 ± 214 (4)	75 ± 26 (8)	36 ± 19 (8)
3% Casein 5% olive oil	1461 ± 131 (4)	152 ± 54 (8)	40 ± 7 (4)
20% Zein, no fat	1878 ± 260 (4)	89 ± 27 (6)	67 ± 9 (4)
20% Casein no fat	2755 ± 573 (6)	101 ± 11 (6)	65 ± 32 (6)
20% Casein 5% olive oil	3353 ± 218 (8)	221 ± 100 (8)	71 ± 19 (8)
20% Casein 10% olive oil	—	215 ± 35·4 (8)	—
20% Casein 10% herring oil	2715 ± 1456 (8)	228 ± 50 (8)	51 ± 13 (8)
20% Casein 10% coconut oil	—	162 ± 21 (4)	—
41B Stock pellets	3297 ± 1600 (8)	100 ± 22 (13)	77 ± 34 (13)

Results are expressed as means ± S.D. of the values obtained from the number of rats assayed which is in parentheses. Each assay was the result of duplicate determinations carried out as described in the section on Methods.

Rats were fed diets for at least 10 days before death. Body weight at death was 160–200 g for all groups except those fed the no-fat no-protein diet, 3% Casein 5% olive oil diet and 20% Zein diet, which weighed 100–140 g. The protein content for stock pellet fed rats was 151 ± 11 mg/g in liver, 151 ± 16 mg/g in kidney, and 107 ± 12 mg/g in lung. In rats fed the other diets, kidney and lung did not differ significantly in protein content; however, the liver protein content fell to 116 ± 7 mg/g in rats fed the no fat no protein diet.

In making these diets the protein composition was varied at the expense of carbohydrate. It could be that the decrease in AHH activity observed in rats fed the 3% casein diet was not due to protein deficiency *per se* but rather to a "glucose repression" effect owing to an increased carbohydrate intake.[16] However, a diet containing 20% zein, a protein deficient in three essential amino acids especially tryptophan,[17] was not as effective as 20% casein in raising AHH activity in the liver in spite of similar carbohydrate content of the two diets. This suggests that essential amino acid intake

rather than "glucose repression" is the controlling factor in the changes of liver AHH activity produced by variation of dietary protein intake.

In the kidney the level of AHH activity in rats fed the no fat no protein diet was unaltered in comparison to that found on feeding the 20% casein no fat diet. The most striking alteration in the level of rat kidney AHH is brought about by the inclusion of natural oils into the diet.

Coconut oil is a highly saturated fat, containing practically no essential unsaturated acids while olive oil is moderately unsaturated and herring oil highly unsaturated. The potentiation by fats of induction of liver cytochrome P-450 by phenobarbitone, depends on their degree of unsaturation and peroxidation.[4] However, the effect of dietary fat in increasing kidney AHH activity does not depend on these factors, but seems to reflect simply total dietary fat intake.

Table 1 shows that there is a significant increase in kidney AHH activity of rats fed 20% casein diets including 10% olive oil, 10% herring oil or 10% coconut oil as compared to rats fed a 20% casein no-fat diet ($P < 0.01$ in all cases). The stock diet 41B contains 3% fat[18] and does not increase the level of kidney AHH. The feeding of these natural oils caused no increase in either the level of liver or lung AHH (Table 1).

Even when a low protein (3% casein 5% oilve oil) diet is fed, the presence of dietary olive oil is able to increase the level of kidney AHH to a higher level than found in rats fed the stock diet 41B ($P < 0.01$).

This increase in activity of kidney AHH by dietary oils is a relatively slow process, occurring maximally after 6–8 days feeding of the diet, (Table 2), as compared to the induction of AHH in liver, kidney and lung by polycyclic hydrocarbons which is maximal within 2 days[19] or as compared to the induction of liver cytochrome P-450 by phenobarbitone which is nearly maximal after 4 days of oral administration,[20] and this would suggest that the increase in the level of kidney AHH is not due to the presence of an inducer in the oils. In addition, the presence of an endogenous inducer would surely increase the level of liver AHH, an effect which was not observed.

TABLE 2. THE TIME COURSE FOR THE INCREASE OF KIDNEY AHH BY DIETARY OILS

Days of feeding diet	AHH activity in different dietary conditions expressed as pmoles 3-hydroxy b.p. formed/30 min/mg protein			
	20% Casein no fat	41B Stock pellets	20% Casein 10% herring oil	20% Casein 10% olive oil
2	103 ± 17	94 ± 17	102 ± 14	94 ± 18
4	98 ± 22	70 ± 19	92 ± 19	93 ± 18
6	123 ± 49	109 ± 48	132 ± 36	218 ± 58
8	124 ± 48	103 ± 9	203 ± 35	207 ± 22
10	91 ± 8	80 ± 9	200 ± 24	215 ± 35

Groups of four rats were fed the diets for the number of days stated and were allowed food *ad lib.* up to the time of death, which was between 10.00 and 11.00 hr. Results are expressed as the mean ± S.D. of individual values.

The dose–response relationship for the increase in activity of kidney AHH by feeding olive oil is shown in Table 3, and this shows that a significant rise in kidney AHH activity is found when the olive oil content of the diet reaches 4 per cent. Once the level of 5 per cent has been passed the increase is maximal.

TABLE 3. DOSE–RESPONSE RELATIONSHIP FOR THE INCREASE OF RAT KIDNEY AHH FEEDING A 20% CASEIN DIET WITH VARYING CONCENTRATIONS OF OLIVE OIL

	% Olive oil concn in 20% casein					
	0	1	2	3	4	5
AHH activity pmoles 3-hydroxy b.p. formed/30 min/mg protein	101 ±11	75·0 ±12	86·0 ±7	107 ±14	151 ±26	221 ±100

Groups of four rats were fed the diets for 10 days and were allowed food *ad lib.* up to the time of death, which was between 10.00–11.00 hr. Results are expressed as the mean ± S.D. of individual values.

DISCUSSION

Our results show that the activity of AHH in the liver and lung, but not kidney, is decreased by feeding rats on a no- or low-protein diet and this observation extends the previous findings that hepatic AHH and pyramidon demethylation[2,21] and cytochrome P-450[20] are decreased in rats fed low protein diets.

In the present study, we have observed that the feeding of a no-protein or 3% casein diet decreases the activity of liver AHH to one third of the activity found in rats fed the stock diet (MRC 41B) when expressed per mg of protein. If AHH activity is expressed on a basis of wet weight of tissue then the activity found in the liver on feeding low protein diets is 25 per cent of that found in rats fed stock-pellets (MRC 41B) or 20% casein diets. The magnitude of these changes is compatible with the reduction in hepatic cytochrome P-450 observed under the same dietary conditions by Marshall and McLean,[20] and the decrease in AHH activity found by Alvares *et al.*[22]

In contrast to liver and lung, the level of AHH activity in rat kidney is not affected by feeding low protein diets but is increased by feeding dietary fat. Jakobsson *et al.*[23] have observed 2–3 fold increases in cytochrome P-450 in rat kidney, but not liver, when rats were fed a stock diet supplemented with 10% lauric acid.

The increase in the level of AHH activity and possibly other cytochrome P-450 associated enzyme activities in kidney caused by the inclusion of fat into the diet, even a low protein diet, will undoubtably have a marked effect on the pharmokinetics of many foreign compounds. This is especially true when a low protein intake decreases the level of the hepatic cytochrome P-450 and associated enzyme activities. Such a mechanism may have contributed to the observation that when rats are fed a 3% casein 5% olive oil diet there is an increase in kidney tumours produced by dimethylnitrosamine (DMN) as compared to rats fed stock diets.[24,25] These authors concluded that the increased renal carcinogenesis observed was the result of an increased proportion of the dose of DMN being metabolized by the kidney microsomal system due to the depression of the liver DMN demethylase activity by the low protein (3% casein 5% olive oil) diet; however, in view of the evidence presented in this report it is possible that an increase in the kidney DMN demethylase activity by the fat component of the diet contributed to the increased renal carcinogenesis observed.

Acknowledgement—This work was supported by the British Nutrition Foundation and Cancer Research Campaign.

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