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GENETIC DIFFERENCES IN THE INDUCTION OF ARYL HYDROCARBON HYDROXYLASE AND ITS COMPONENTS BY 3-METHYLCHOLANTHRENE IN LIVER AND LUNG MICROSOMES AMONG FOUR STRAINS OF GUINEA PIGS

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Abstract—Four strains of guinea pigs (Hartley, No. 2, No. 13 and JY-1) were examined for the effects of intraperitoneal treatment with 3-methylcholanthrene on aryl hydrocarbon hydroxylase activity, total cytochrome P-450 content in liver and lung microsomes, and NADPH–cytochrome *c* reductase activity in liver microsomes. Following treatment with 3-methylcholanthrene at a dose of 50 mg/kg body weight, aryl hydrocarbon hydroxylase activity and cytochrome P-450 content in liver were both increased in all the strains used, and the activity of NADPH–cytochrome *c* reductase in liver was also increased in all strains except No. 13. While the cytochrome P-450 content in lung was increased in all the strains except No. 13, there was no increase in the aryl hydrocarbon hydroxylase activity in lung from any strain of guinea pig examined. When the dose of 3-methylcholanthrene was increased to 250 mg/kg body weight, an apparent induction of aryl hydrocarbon hydroxylase was detected in the lung from the Hartley strain of guinea pigs, but not in the other three strains. In summary, marked differences were seen in sensitivity to 3-methylcholanthrene between liver and lung, and apparent strain differences were observed among the guinea pigs used in this experiment.

Guinea pigs are known to be resistant to some kinds of chemical carcinogens such as 2-acetylaminofluorene [1, 2], 3′-methyl-4-dimethylaminoazobenzene [3], and nitrosamines [4]. Toth [5], however, stated that the guinea pig was found to be at least as susceptible as the mouse to the stimulus of 7,12-dimethylbenz[*a*]anthracene. In liver of guinea pigs, the low inducibility of cytochrome P-450 and the consequent inability of this species of animals to activate 2-acetylaminofluorene to bind DNA [6] and the relative proficiency in the capacity for detoxication of active metabolites, such as reduction of *N*-hydroxy-2-acetylaminofluorene [7], could be responsible for the resistance to this aromatic amide. Such explanations, however, have not proved generally applicable, because guinea pig liver is efficient in *N*-hydroxylating 2-acetylaminofluorene [8] and 4-aminobiphenyl [9] *in vitro*.

In mice, there are strain differences in the susceptibilities to carcinogens such as polycyclic aromatic hydrocarbons and in the inducibilities of aryl hydrocarbon hydroxylase (AHH)† by treatment with 3-methylcholanthrene (MC) in some tissues such as liver and lung, and a correlation was observed clearly between susceptibility to the carcinogens and AHH inducibility [10–13]. Thorgeirsson *et al.* [29] also showed the association of mutagenicity *in vitro* of 2-acetylaminofluorene and other acylaminofluorenes with the inducibility of *N*-acetylarylamine *N*-hydroxylase by aromatic hydrocarbon in genetically different strains of mice.

In the present study, we examined whether AHH is inducible or not in liver and lung microsomes from four strains of guinea pigs. At the same time, we followed changes in the content of cytochrome P-450 and in the activity of NADPH–cytochrome *c* reductase (f_{PT}), both of which are responsible for the expression of AHH activity in microsomes.

MATERIALS AND METHODS

Animals. The Hartley strain of guinea pigs, bred in a closed colony, and the JY-1 inbred strain were supplied by the Tokyo Experimental Animal Co. Ltd., Tokyo, Japan. Inbred No. 13 (ST13IN) and No. 2 (ST2IN) strains of guinea pigs were obtained from the Nippon Institute of Biological Science Co. Ltd., Tachikawa, Japan. The JY-1 strain of guinea pigs was a subline of the Hartley strain which was derived from Rockland Farms (U.S.A.), originally, transferred to the National Institute of Health of Japan in 1959, and established as an inbred strain of guinea pigs. No. 2 and No. 13 strains of guinea pigs originated from the National Institutes of Health, U.S.A., and were transferred to the National Institute of Health of Japan in 1969. Body weights of animals used in this study were 250–300 g.

Chemicals. The sources of chemicals and reagents used were as follows: horse heart cytochrome *c*, from the Sigma Chemical Co., St. Louis, MO, U.S.A.; tetrasodium salts of NADPH and NADH, from the Oriental Yeast Co. Ltd., Tokyo, Japan; 3-hydroxybenzo[*a*]pyrene, from Dr. N. Kinoshita, Kyushu University, Fukuoka, Japan; and the

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† Abbreviations: AHH, aryl hydrocarbon hydroxylase; MC, 3-methylcholanthrene; and f_{PT}, NADPH–cytochrome P-450 (cytochrome *c*) reductase.

remainder, from the Wako Pure Chemical Co. Ltd., Osaka, Japan.

Treatment of animals and preparation of microsomes. All animals were housed in equipment that was conditioned for air, light and temperature, and were fed with vitamin C-containing animal chow (Oriental RC-4) and water *ad lib.* for at least 1 week before the experiment began. MC was dissolved in corn oil and was injected intraperitoneally every 24 hr into the animals at the dose indicated in each experiment. Twenty-four hours after the final injection, the animals were killed, and the livers and lungs were excised immediately. The liver from each animal was separately homogenized in 1.15% (w/v) KCl solution, containing 1 mM EDTA, in a Potter–Elvehjem type of glass homogenizer with a Teflon pestle, and the microsomes were prepared, by the differential centrifugation method, from the individual homogenates. The lungs were homogenized by "Ultra Turrax" (IKA-WERK), which is a motor-driven tissue homogenizer with a spinning blade. Microsomes from lung homogenates were prepared with $CaCl_2$ by the method of Schenkman and Cinti [14] because, after the administration of MC, detectable increases in the level of cytochrome P-450 and AHH activity were observed in the lung microsomes of guinea pig prepared by this method, as well as in those prepared by the conventional centrifugation method used for the preparation of liver microsomes (data not shown). The precipitated microsomes were suspended in 100 mM potassium phosphate buffer (pH 7.25) containing 20% (v/v) glycerol and were stored at −80° until use. The animals in the control groups were not treated and were killed at about the same time as were the MC-treated animals.

Assay procedures. AHH activity was assayed by the method of Nebert [15]. Cytochrome P-450 content and f_{PT} activity in liver microsomes were determined by the method described by Omura and Sato [16], and by Omura *et al.* [17] respectively. The cytochrome P-450 content in lung microsomes was measured from the difference spectrum of dithionite-reduced minus non-reduced CO–hemoprotein complex with the use of ascorbic acid and phenazine methosulfate to avoid interference due to contamination by hemoglobin [18]. For comparison between the method of Omura and Sato and that of Johannesen and DePierre for assay of cytochrome P-450, liver microsomes of guinea pigs were used. As shown in Table 1, apparent induction of cytochrome P-450 after the administration of MC was observed when the assay was performed by both methods mentioned above, although the values obtained by Omura's method were higher than those by Johannesen's method. Protein concentration was determined by the method of Lowry *et al.* [19], with bovine serum albumin as the standard. Specific enzyme activities and contents were all expressed per mg of microsomal protein by their mean value and the standard error of the mean. Statistical analyses were performed by Student's *t*-test. A significant difference was defined as a P value of less than 0.05.

RESULTS

Effect of 3-methylcholanthrene in vivo in liver. We first studied the effects of different doses of MC on enzyme activities and cytochrome P-450 content in liver microsomes from the JY-1 strain of male guinea pigs. Figure 1 shows that AHH activity, cytochrome P-450 content and f_{PT} activity were all increased with statistical significance in the animals treated twice with MC at a dose of 25 mg·(kg body wt)$^{-1}$·day^{-1}, when compared with those in the non-treated animals. On the other hand, no appreciable difference in these dose–response relationships was observed between the male and female animals in liver microsomes (data not shown).

From these data, we decided to compare non-treated female animals and female animals treated twice with MC in terms of enzyme activities and cytochrome P-450 content in the following experiments.

Strain differences in liver. Figure 2 shows the effects of treatment with MC on AHH activity, cytochrome P-450 content and f_{PT} activity in the four strains of guinea pigs: Hartley, No. 2, No. 13 and JY-1. All experiments were performed twice and values estimated for each group were calculated from a total of six to eight guinea pigs. AHH activity and cytochrome P-450 content in the liver were both increased with statistical significance in all four strains of guinea pigs by treatment with MC. In the case of f_{PT}, there were also found to be statistically significant increases in the livers of the Hartley, No. 2 and JY-1 strains of guinea pigs, but not in the No. 13 strain.

Strain differences in lung. AHH activity and cytochrome P-450 content were examined in lung micro-

Table 1. Comparison of the two different methods of assay for cytochrome P-450 content of liver from female guinea pigs of the Hartley strain*

Treatment	Cytochrome P-450 content (nmoles/mg protein)	
	Method of Omura and Sato	Method of Johannesen and DePierre
None	0.795 ± 0.045 (4)	0.586 ± 0.036§ (4)
3-Methylcholanthrene†	1.12 ± 0.02‡ (4)	0.910 ± 0.024‡,§ (4)

* Values are means ± S.E.M. (N).

† 3-Methylcholanthrene was used at a dose of 125 mg·day^{-1}·(kg body wt)$^{-1}$ for 2 consecutive days.

‡,§ Significantly different from its own control and from the value in the conventional method of Omura and Sato, $P < 0.05$.

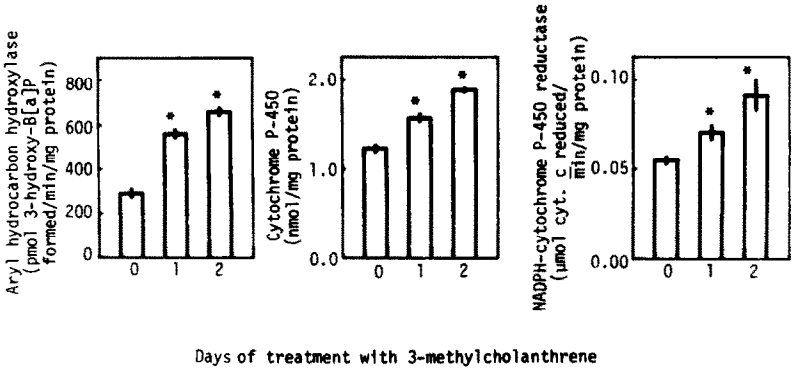

Fig. 1. Effects of 3-methylcholanthrene on aryl hydrocarbon hydroxylase activity, cytochrome P-450 content, and NADPH–cytochrome *c* reductase activity in liver microsomes from the male guinea pigs of the JY-1 strain. 3-Methylcholanthrene was used at a dose of 25 mg·(kg body wt)$^{-1}$·day^{-1}. The vertical line on the top of each column represents the standard error of the mean. An asterisk indicates a significant difference from control, $P < 0.05$.

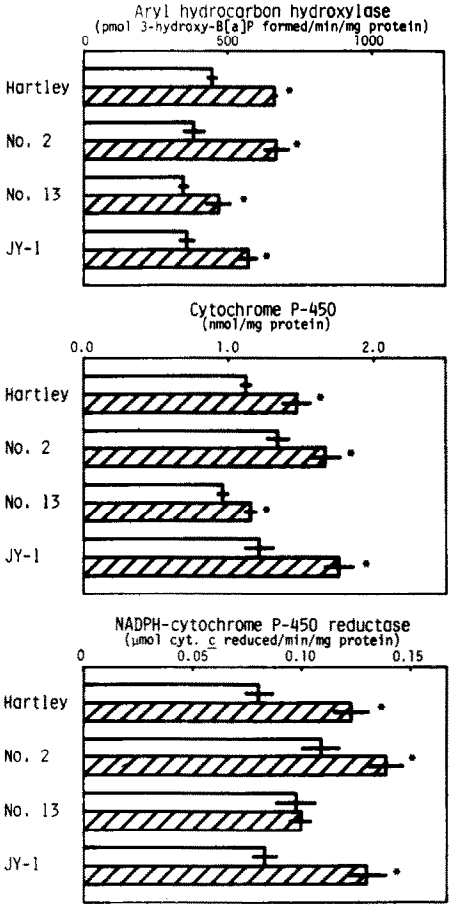

Fig. 2. Strain difference in the effects of 3-methylcholanthrene on aryl hydrocarbon hydroxylase activity, cytochrome P-450 content, and NADPH–cytochrome *c* reductase activity in liver microsomes from female guinea pigs. The open columns represent the values of the control groups, and the hatched columns, of the 3-methylcholanthrene-treated groups. 3-Methylcholanthrene was used at a dose of 25 mg·(kg body wt)$^{-1}$·day^{-1} for 2 consecutive days. An asterisk indicates a significant difference from control, $P < 0.05$.

somes from the same guinea pigs that we used for the assay of liver microsomes. Figure 3 shows that treatment with MC apparently increased the cytochrome P-450 content in all strains except No. 13. No increases in AHH activity, however, were observed in any of the four strains. The inducibility, after the administration of MC, of the AHH activities of liver and lung was different.

To elucidate whether or not AHH activity in lung is refractory to MC treatment, relatively higher doses of MC were administered to guinea pigs of the Hartley strain. AHH activity was clearly increased with higher doses of MC, such as 100 and 250 mg, as shown in Table 2. There was, however, no significant difference in the cytochrome P-450 content between the control and treated animals, and this refractoriness was apparently in contrast to the results using a low dose of MC (Fig. 3).

Next, using MC treatment at a dose of 250 mg, the presence or absence of strain differences was examined in lung microsomes from the four strains of guinea pigs. Figure 4 shows that, except for the Hartley strain, no increase was observed in AHH activity in the strains of guinea pigs used, indicating

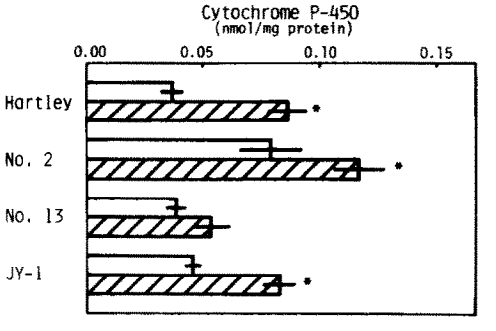

Fig. 3. Strain differences in the effects of 3-methylcholanthrene on cytochrome P-450 content in lung microsomes from female guinea pigs. All the guinea pigs were the same animals that were used for the assay of liver microsomes, indicated in Fig. 2, and were treated with 3-methylcholanthrene at a dose of 25 mg·(kg body wt)$^{-1}$·day^{-1} for 2 consecutive days. An asterisk indicates a significant difference from control, $P < 0.05$.

Table 2. Effect of 3-methylcholanthrene treatment on aryl hydrocarbon hydroxylase activity and cytochrome P-450 content of lung from female guinea pigs of the Hartley strain*

Dose of MC (mg/kg body wt)	Aryl hydrocarbon hydroxylase†	Cytochrome P-450‡
0	19.8 ± 3.8 (4)	0.0835 ± 0.0188 (3)
100	33.0 ± 3.6§ (4)	0.0953 ± 0.0114 (4)
250	49.8 ± 10.8§ (4)	0.0801 ± 0.0112 (4)

* Guinea pigs were treated with half of the dose shown in the table for 2 consecutive days intraperitoneally.

† Aryl hydrocarbon hydroxylase activity is expressed as pmoles 3-hydroxybenzo[*a*]pyrene formed·min^{-1}·(mg protein)$^{-1}$ ± S.E.M. (N).

‡ Cytochrome P-450 content is expressed as nmoles/mg protein ± S.E.M. (N).

§ Significantly different from its own control, $P < 0.05$.

an apparent strain difference in the inducibility of AHH. On the other hand, no induction of cytochrome P-450 was detected in the four strains of guinea pigs (data not shown) as shown for the Hartley strain in Table 2.

Carbon monoxide-complex difference spectra of reduced hemoproteins. Figure 5 shows the CO-complex difference spectra of dithionite-reduced hemoproteins of liver microsomes from the non-treated and MC-treated animals. Apparently there was a blue shift of the Soret maximum by about 2 nm in the microsomes from the MC-treated animal, compared with the Soret maximum from the non-treated animal, suggesting the induction of cytochrome "P-448" in the liver microsomes by treatment with MC. These data are slightly different from the results of Kawajiri *et al.* [6], in which was shown the lack of a blue shift in guinea pig liver after the administration of MC. On the other hand, no blue shift in the carbon monoxide-complex difference spectra of reduced hemoprotein in lung microsomes was observed even after the administration of MC (data not shown).

DISCUSSION

The inducibility of AHH by treatment with MC in liver and lung of mice has been found to correlate well with the susceptibility of the species to certain kinds of chemical carcinogens such as polycyclic aromatic hydrocarbons [10–12]. This correlation could be explained on the basis that the microsomal monooxygenase system played a critical role in the activation step of carcinogens in the tissues. We, therefore, examined inducibility of microsomal AHH in guinea pigs, a species known to be resistant to some kinds of chemical carcinogens [1–4]. Though there have been no experiments studying strain differences in the susceptibility of this species to chemical carcinogens, we sought the presence of strain differences in AHH induction. The low inducibility by MC of AHH in liver, and its lack in lung, from the Hartley strain of guinea pigs have been reported previously [20, 21].

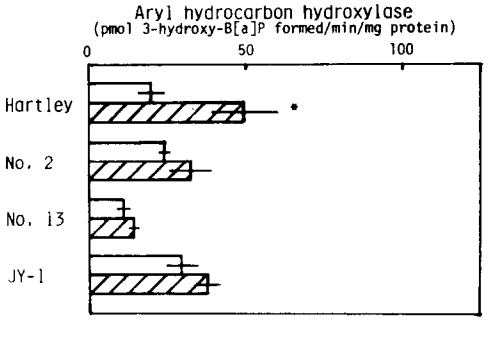

Fig. 4. Strain differences in the effects of 3-methylcholanthrene on aryl hydrocarbon hydroxylase activity in lung microsomes from female guinea pigs. 3-Methylcholanthrene was used at a dose of 125 mg·(kg body wt)$^{-1}$·day^{-1} for 2 consecutive days. All the expressions are the same as in Fig. 3.

In the experiments reported here, a similar inducibility of the liver microsomal AHH by MC was seen in all four strains: Hartley, No. 2, No. 13 and JY-1 (Fig. 2). But the induced levels of liver AHH activities in the four strains were much lower than those in the so-called "responsive" strains of mice, even though the same basal levels of activity were observed in both species [10–12]. The content of cytochrome P-450, one of the crucial components of the microsomal monooxygenase system, also increased in all four strains of guinea pigs after MC treatment (Fig. 2) to a degree similar to that in mice [22]. Another component, f_{PT}, which is known not to be induced in mice or in rats by MC [23], was induced too in these guinea pigs, except in the No. 13 strain (Fig. 2). The fact that a lower degree of AHH induction was detected in these four strains of guinea pigs, compared with the "responsive" strains of mice, may result from a low catalytic activity of the cytochrome "P-448" for benzo[*a*]pyrene hydroxylation, because a cytochrome "P-448" with a low catalytic activity for benzo[*a*]pyrene hydroxylation has also been found in rabbit liver [24]. On the other hand, it is of interest that in rat liver and lung the induced cytochrome "P-448" has remarkably high AHH activities in the microsomal and reconstituted systems [25, 26]. Of course there is a possibility that some other factors, such as a special environment of the microsomal membrane and special mutual interactions of each component of the monooxygenase system in the microsomal membrane of liver, are responsible for showing the low AHH activity even if the induced cytochrome "P-448" is present. To determine pre-

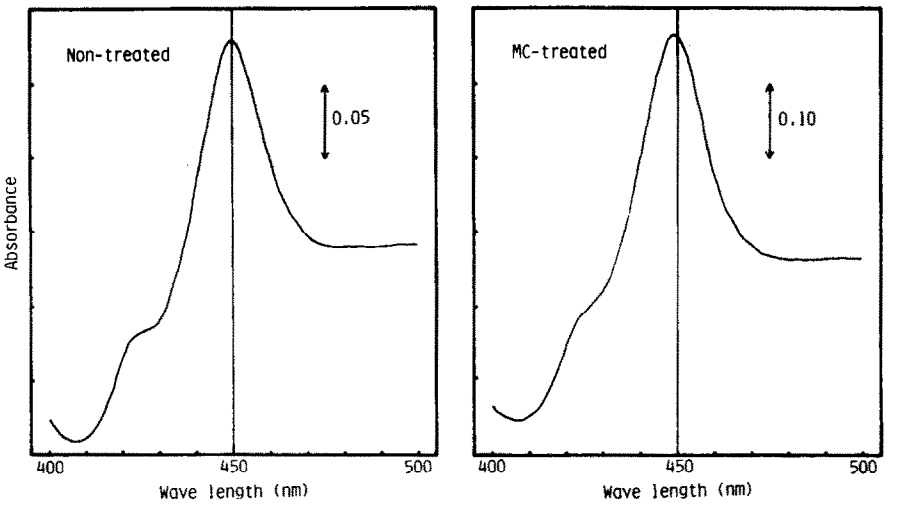

Fig. 5. Carbon monoxide-complex difference spectra of reduced hemoproteins of liver microsomes from non-treated and 3-methylcholanthrene-treated female guinea pigs (Hartley strain). The spectrum from the non-treated guinea pig is shown on the left side of this figure (protein concentration, 1.12 mg/ml), and that from the 3-methylcholanthrene-treated guinea pig, on the right side (protein concentration, 1.48 mg/ml).

cisely the catalytic activity of this cytochrome "P-448" in guinea pig liver, the purification of this cytochrome is required.

Negishi and Nebert [27] and Nebert *et al.* [28] mentioned that, in mice pretreated with MC, there were two kinds of cytochrome P-450, namely, cytochrome P_1-450, which shows no blue shift and a high catalytic activity, and cytochrome P-448, which shows a blue shift of 2 nm and a relatively low catalytic activity for benzo[*a*]pyrene hydroxylation, and that the induction of AHH and cytochrome P_1-450 occurs in neonatal rabbit liver and lung but not in adult rabbit liver and lung, but that cytochrome P-448, however, is present in adult rabbit liver and lung. Liver cytochrome P-448 from the guinea pigs used shows a character seemingly similar in catalytic activity to the cytochrome P-448 in rabbit and mouse mentioned above.

In lung, all strains of guinea pigs, except No. 13, treated with a low dose (50 mg/kg body wt) of MC, exhibited the apparent induction of the microsomal cytochrome P-450 (Fig. 3), but no AHH induction was found in any of the strains of animals examined, as reported previously by Bilimoria *et al.* [21]. When the total dose of MC applied was increased up to 250 mg/kg of body weight, there appeared increases in AHH activity in the lung microsomes from the Hartley strain of guinea pigs, compared with the control, but not in the other three strains, indicating an apparent strain difference in AHH induction. Furthermore, cytochrome P-450 did not show any increase in the lungs from all strains of guinea pigs, even after such a high dose of MC was administered, indicating an apparent discrepancy between the induction of cytochrome P-450 and AHH induction in the lung. It is of importance to note from this experiment that there are marked differences between liver and lung in the sensitivity to induction of AHH with MC, and that the No. 13 strain of guinea pigs is markedly different from the other three strains of guinea pigs in the inducibility of cytochrome P-450 in the lung of f_{PT} in the liver, showing a strain difference in the guinea pigs used.

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