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Strain- and Organ-Dependent Differences in Induction of Aryl Hydrocarbon Hydroxylase Activity by 3-Methylcholanthrene

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Several polyaromatic hydrocarbons are suspected to be carcinogenic and may be a major cause of the human lung cancer. They are present in cigarette smoke and in waste material whenever organic matter is burned with an insufficient amount of oxygen. In the murine tissues, liver, lung, kidney, small intestine, lymphocytes etc., the first step of the metabolism of polyaromatic hydrocarbons involves the enzyme aryl hydrocarbon hydroxylase (AHH), which is known as a component of the microsomal mixed function oxidase system dependent on NADPH and cytochrome P-450 (P-450)(Conney 1967; Nebert and Gelboin 1968a, 1968b, 1969; Lawrence and Aristo 1983). Hepatic and extrahepatic AHH is induced by polyaromatic hydrocarbons such as 3-methylcholanthrene (MC) and the inducibility is under the control of the aromatic hydrocarbon (Ah) locus; certain inbred strains of mice are susceptible of AHH induction by MC treatment (Ah responsive strains), while other inbred strains are not (Ah non-responsive strains). Therefore, the inheritable inducibility of AHH may be an important factor in carcinogenesis due to polyaromatic hydrocarbons (Nebert et al. 1974).

The objective of this study is to understand more fully strain- and organ-dependent differences in inducibility of AHH by MC with the genetically established mouse strains as materials. Also the AHH levels of blood lymphocytes and splenic lymphocytes are compared with those of several freshly excised organs of the strains of mice.

MATERIALS AND METHODS

MC and benzo(a)pyrene (BP) were purchased from Sigma Chemical Co. (St. Louis, Missouri) and the latter was purified as previously described (Nagayama et al. 1983). RPMI-1640 and lympholyte M were obtained from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan) and Cedarlane Laboratories Ltd. (Horby, Ontario, Canada), respectively. 3-Hydroxybenzo(a)pyrene (3-OHBP) was provided by

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Inbred strains of mice used were C_{57}BL/6J (C_{57}), C_3H/HeN (C_3H), CBA/J (CBA), A/J, BALB/c/Slc (BALB/c), DDD:Qdj (DDD), DBA/J/Sea (DBA), NZB/Sea (NZB), NZW/Sea (NZW) and AKR/J/Sea (AKR). Mice were kept in a temperature- and humidity-controlled room (22 ± 1°C , 55 ± 5% RH) maintained on a diurnal cycle of 12 hr light/12 hr darkness and permitted water and food (CE-2, Nippon Clea Co.,Ltd., Osaka, Japan) ad libitum. At 8 weeks of age, mice were given a single i.p. injection of MC at a dose of 42 mg/kg of body weight. Control mice received vehicle (olive oil) alone in a same volume (8 ml/kg of body weight) and on the same time schedule as above. After 3 days, the mice were sacrificed by cervical dislocation. The livers, kidneys and lungs were excised, rinsed with an ice-cold 0.02 M N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid buffer (adjusted with KOH to pH 7.4 and mixed with 0.15 M KCl), weighed, minced and homogenized in the above buffer using a Potter-Elvehjem homogenizer with a Teflon pestle (9-fold volume (v/w) of the buffer for liver; 2 ml and 1 ml of the buffer for a pair of kidneys and a whole lung, respectively). The homogenates were centrifuged at 9,000g at 4°C for 15 min; the 9,000g supernatants (S-9) were used for the AHH assay. The reaction mixture (total volume 1.05 ml) contained 125 μmoles $MgCl_2$, 100 μmoles BP in 50 μl methanol and S-9 (Nebert and Gelboin 1968b). The latter from the liver was used in 0.02 ml, from the kidney 0.5 ml and from the lung 0.25 ml. Incubation was performed at 37°C for 5 min (liver and lung) or 20 min (kidney). The reaction was stopped by addition of 1 ml of cold acetone. The solution was then added with 3.5 ml n-hexane and centrifuged at 3,000 rpm for 2 min. Aliquots of the organic phase (0.5 ml for liver and lung, 2 ml for kidney) were each extracted with 2.0 ml of 1 N NaOH. AHH activity was determined at an excitation wavelength of 395 nm and an emission wavelength of 522 nm using a Hitachi spectrophotofluorometer (Model 650-10S, Hitachi Ltd., Tokyo, Japan). Further details of the method were described previously (Nagayama et al. 1983). The enzyme activity was expressed as the formation of hydroxylated metabolites with a fluorescence equivalent to 1 pmole 3-OHBP per min per mg protein(the lower limit of detection: 0.001 pmole/min/mg). Protein concentration was determined according to Lowry et al. (1951), bovine serum albumin being used as standard. Statistical differences among AHH values were determined by Student's t-test.

To prepare lymphocytes, blood was collected from 2 groups (5 control; 5 MC-treated) of mice through the inferior vena cava shortly after killed by cervical dislocation. Blood lymphocytes were separated from 5 ml (from 5 mice) of heparinized venous blood using a lympholyte M, washed twice, and finally suspended in an RPMI-1640 medium. Cultures containing 10^6 cells per ml were set up. The medium was added with heat-inactivated fetal calf serum, 1% phytohemagglutinin, 1% pokeweedmitogen, 100 IU penicillin and 100 μg streptomycin per ml. Spleens from 5 mice

at one batch were cut with scissors and gently teased with a slide glass in the RPMI-1640 medium. To sediment large tissue debris, cell suspensions were transferred to conical tubes and stood for 2-3 min in crushed ice. The supernatants were centrifuged at 4°C at 1,500 rpm for 5 min. The pellets were then washed with 0.17 M ammonium chloride in 0.1 M Tris-HCl buffer, pH 7.2, to remove red blood cells and washed twice with the RPMI-1640 medium by centrifugation as above. The cells were finally suspended in a complete medium (the RPMI-1640 medium supplemented with 20% fetal calf serum) containing 0.1% concanavaline A and incubated at 37°C at humid atmosphere of 5% CO_2 in air. After 48 hr, MC dissolved in acetone was added to the culture at the final concentration of 2.5 μM. Control samples were treated with acetone only. Incubation was continued for additional 48 hr. Then, AHH activity was measured according to the method of Gurtoo et al. (1975) with a modification. Cells were harvested by centrifugation, washed and suspended at a density of 1-4 x 10^6 cells per ml in 0.05 M Tris-HCl buffer, pH 8.5, containing 3 mM $MgCl_2$, 0.2 M sucrose, 1.7 mM NADPH and 1.3 mM NADH. Benzo(a)pyrene (80 μM) was added to the cell suspension. After incubation at 37°C for 50 min (Nagayam et al. 1985a, 1985b), the suspension received 4.5 ml of acetone/hexane (1:3.5) mixture. Aliquots of the organic phase was extracted with 1.0 ml of 1 N NaOH and examined for fluorescence assay by using authentic 3-OHBP as a standard. The values were expressed as 3-OHBP moles per 10^6 cells per min (the lower limit of detection: 0.001 pmole/min/10^6 cells).

RESULTS AND DISCUSSION

In Ah non-responsive strains of mice, a single administration of MC at a dose of 42 mg/kg of body weight produced little statistically significant changes in weight of the whole body and of the liver, kidney and lung between the MC-treated and the control mice. On the other hand, significant decreases in weight of the above items compared to the control were seen in Ah responsive strains (data not shown). Differences were also clearly observed in hepatic AHH activities (Table 1); the values in Ah responsive strains were significantly increased after a single administration of MC, whereas those in Ah non-responsive strains were not. Induced activities were normalized with the control (thus basal) activities (cf the AHH inducibility column). The basal AHH activity of the 10 inbred strains were between 60 and 200. As to the inducibility, the value of A/J mouse was the lowest in the responsive strains but it was significantly predominated over the mean of the Ah non-responsive strains. Among the Ah non-responsive strains, NZB gave a high inducibility but the value was lower than the mean of the responsive strains. On the whole, the AHH inducibility of the Ah responsive strains were higher than that of the non-responsive strains.

We then examined the levels of AHH activity in the lung, kidney and blood lymphocytes and splenic lymphocytes (Table 2). The activities were thoroughly lower than those of the liver. The distribution of the basal activity among the different organs and

Table 1. Hepatic AHH activity in Ah responsive and Ah non-responsive strains after administration of 3-methylcholanthrene

Mouse strain	AHH activity[a] Control (A)	AHH activity[a] MC-induced (B)	AHH inducibility (B/A)
Ah responsive			
C_{57}(10)[b]	219.52 ± 8.95[c]	2457.49 ± 208.64	11.19
CBA(10)	83.90 ± 5.08	781.17 ± 59.89	9.31
C_3H(10)	86.75 ± 3.73	764.41 ± 71.71	8.81
BALB/c(10)	137.58 ± 14.96	1098.00 ± 82.90	7.98
A/J(10)	99.21 ± 7.16	347.60 ± 32.79	3.50
Mean	125.4 ± 25.4	1089.8 ± 362.1	8.3 ± 1.3
Ah non-responsive			
DDD(10)	130.28 ± 6.06[c]	128.10 ± 7.82	0.98
DBA(10)	112.45 ± 6.31	94.54 ± 7.75	1.19
AKR(10)	58.96 ± 4.85	83.72 ± 8.26	1.42
NZB(10)	66.13 ± 7.79	136.40 ± 8.60	2.06
NZW(10)	137.08 ± 13.35	634.08 ± 72.81	4.63
Mean	101.0 ± 16.2	215.4 ± 105.1	2.1 ± 1.3

[a]Expressed in terms of 3-hydroxybenzo(a)pyrene (pmoles/min/mg protein) formed.
[b]Figures in parentheses indicate the number of mice used.
[c]Each value represents the mean ± S.E.

cells were somewhat similar between the Ah responsive and non-responsive strains. The levels of activity and the values of inducibility (MC-induced AHH activity / control AHH activity) were low in the lymphocytes but higher in the organs tested. Increase in AHH activity occurred also in organs or cells other than the liver. In particular, a marked induction was observed in the kidney where inducibility was 2.1 times higher than in the liver (compare the means of the strains, Tables 1 and 2). From the data described above, the ratios of the different extra-hepatic AHH inducibilities toward the hepatic AHH inducibility (termed relative inducibilities) were calculated (details omitted). These values were found to serve as indices for the organ- or cell-specific potency of the enzyme induction by MC at a fixed dose. For the kidney and the lung, the mean values of the Ah responsive strains were 2.1 and 0.68, respectively, and those of the lymphocytes were 0.24 (blood) and 0.25 (spleen). Thus we conclude that in the responsive strains the kidney was high in relative inducibility by MC but the lung and lymphocytes were low. On the other hand, the lung showed the value of 1.7 in the Ah non-responsive strains thus indicating a higher relative inducibility than the kidney, the blood lymphocyte and the

Table 2. Basal and MC-induced AHH activities in lung, kidney, blood and splenic lymphocytes

Strain	Treatment	AHH activity[a] Lung[b]	Kidney[b]	Lymphocytes[c] Blood	Spleen
Ah responsive					
C_{57}	Control	6.63 ± 0.68	0.20 ± 0.01	0.010	0.001
	MC	32.88 ± 3.36	3.52 ± 0.25	0.012	0.002
CBA	Control	11.43 ± 0.70	0.63 ± 0.07	0.033	0.006
	MC	60.31 ± 2.76	3.47 ± 0.30	0.050	0.009
C_3H	Control	15.51 ± 0.81	0.09 ± 0.01	0.004	0.002
	MC	94.59 ± 3.97	3.00 ± 0.39	0.006	0.003
BALB/c	Control	10.22 ± 0.97	0.12 ± 0.02	0.021	0.002
	MC	41.59 ± 3.39	3.05 ± 0.13	0.029	0.003
A/J	Control	8.60 ± 0.62	0.52 ± 0.04	0.001	0.001
	MC	28.50 ± 1.95	2.58 ± 0.10	0.002	0.002
Mean	Control	10.48 ± 1.49	0.32 ± 0.11	0.014	0.002
	MC	51.57 ± 12.06	3.12 ± 0.17	0.020	0.005
	(MC/Control)[d]	(5.11 ± 0.64)	(16.91 ± 5.32)	(1.52)	(1.70)
Ah non-responsive					
DDD	Control	10.05 ± 1.18	0.99 ± 0.07	0.008	0.005
	MC	13.90 ± 1.61	1.23 ± 0.08	0.011	0.009
DBA	Control	6.79 ± 0.42	1.75 ± 0.26	0.007	0.002
	MC	15.06 ± 1.30	1.72 ± 0.15	0.007	0.003
AKR	Control	6.19 ± 0.37	0.30 ± 0.04	0.006	0.010
	MC	20.71 ± 1.56	0.50 ± 0.05	0.006	0.013
NZB	Control	7.91 ± 0.39	0.24 ± 0.03	0.011	0.001
	MC	19.05 ± 1.18	0.19 ± 0.02	0.022	0.006
NZW	Control	5.20 ± 0.67	1.23 ± 0.06	0.012	0.009
	MC	38.54 ± 4.05	7.05 ± 0.63	0.021	0.010
Mean	Control	7.23 ± 0.83	0.90 ± 0.29	0.009	0.005
	MC	21.45 ± 4.45	2.08 ± 0.92	0.012	0.008
	(MC/Control)[d]	(3.35 ± 1.06)	(2.08 ± 0.92)	(1.43)	(2.20)

[a]Expressed in terms of 3-hydroxybenzo(a)pyrene (pmoles/min/mg protein or pmoles/min/10^6cells) formed.
[b]Each value represents the mean ± S.E.
[c]Each value represents the mean only because of limited space.
[d]Values in parentheses represents the mean ± S.E. of the whole AHH inducibility (MC/Control).

splenic lymphocyte, whose values were 0.98, 0.86 and 1.31, respectively. This is of interest in reference to the fact that the lung is the target of several carcinogenic polyaromatic hydrocarbons. It appears therefore that in a given strain there was an organ-dependent variation in inducibility, indicating

genes in the Ah locus are expressed in organ-specific manner, and the expression is in turn modified according to the strain.

In the present study the mouse strains A/J and NZW showed an Ah responsiveness against our expectation. In repeated experiments the inclination was reproducible. This is incompatible with the results reported by Nebert et al. (1975), and it is likely that some genetic changes occurred in these strains during keeping. In such strains, it is no more easy to judge whether they are Ah responsive or non-responsive from the AHH inducibility. It should be noted that hepatic AHH inducibility is not a sole factor for Ah responsiveness and others showing difference between Ah responsive and non-responsive strains (Poland et al.1974) should be taken into consideration.

The difference in AHH inducibility between inbred mouse strains like that reported in the present study has also been published previously (Nebert and Jensen 1979). The strain-dependent variation of the mouse is comparable to the range of basal and MC-induced AHH activity and degrees of AHH inducibility observed in different individuals of human (Nagayama et al. 1985a, 1985b).

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