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Organ Specificity of Aryl Hydrocarbon Hydroxylase Induction by Cigarette Smoke

M. Yoshikawa,[1] K. Arashidani,[1] T. Kawamoto,[2] and Y. Kodama[2]

[1]Division of Occupational Hygiene, School of Medical Technology, [2]Department of Environmental Health, School of Medicine, University of Occupational and Environmental Health, Iseigaoka 1-1, Yahatanishi-ku, Kitakyushu-shi, Fukuoka 807, Japan

Cigarette smoke appears to be one of the commonest carcinogenic products of our environment. Numerous epidemiological studies have shown an increase in the incidence of cancer in several organs, notably the lung, among cigarette smokers. Polycyclic aromatic hydrocarbons (PAHs) are among the many of compounds identified in cigarette smoke and contribute to the high incidence of lung cancer in smokers.

Biotransformation of many chemicals found in cigarette smoke, such as PAHs and nitrosamines, is generally considered essential for the mutagenic, carcinogenic effects of these xenobiotics. In fact, the genotic action of these premutagens or precarcinogens is dependent on metabolic activation catalyzed by microsomal monooxygenases(IARC 1986). The first enzymatic reaction of the PAHs metabolic pathway is catalyzed by a cytochrome P-450-dependent monooxygenase (Nebert 1982; Minchin et al. 1983), the aryl hydrocarbon hydroxylase(AHH). AHH leads to the formation of reactive arene oxides, which are further metabolized by enzymatic and non-enzymatic reaction into many metabolites. These reactive intermediates could also bind covalently to cellular macromolecules, and generate a variety of toxic effect inducing mutations and cancers (Gelboin 1980).

AHH induction in laboratory animals exposed to cigarette smoke has also been reported, and the data show that this response is highly dependent on species and tissues(Abramson et al. 1977; Bilimoria et al.1977). Exposure of small laboratory animals to cigarette smoke generally induces AHH in the kid-

Send reprint requests to M. Yoshikawa at the above address.

ney and lung, while the effect of cigarette smoke on the hepatic AHH activity appears variable (Bilimoria et al.1980; Kushinsky et al. 1976; Raunio et al. 1983).

In the present study, two different exposures, single and consecutive ones, of rats to cigarette smoke were performed to examine the relationship between inhalation of cigarette smoke and AHH activity in the liver, kidney and lung.

MATERIALS AND METHODS

Male Wistar rats weighing 170-180 g were obtained from Japan Clea Company, and the rats were acclimated for 3 days before use. The rats received standard rodent feed and tap water *ad libitum*. Two different exposure systems were used in the present study; one was a single exposure, the other was a consecutive exposure. In the single exposure, cigarette smoke was generated under conditions designed to simulate human smoking, drawing a 35 mL puff of 2 sec duration into a chamber(vol. 6L). The rats were exposed to cigarette smoke for 10 min. In this system, 1, 5, 10 or 15 puffs of cigarette smoke were delivered into the chamber for the purpose of examining a dose-response relationship between smoke concentration and AHH activity. One group of rats was exposed to cigarette smoke of 15 puffs for 10 min, and AHH activities in the liver, kidney and lung were measured as a function of time. In the consecutive exposure, various numbers of cigarette(s)/hr were burned in a chamber (vol. 100L) with clean air ventilation (about 15 L/min). The rats were exposed to cigarette smoke of 1, 3 or 5 cigarettes/hr, 8 hrs/day, for 5 consecutive days. One group of rats was exposed to cigarette smoke of 5 cigarettes/hr, for 4 hrs only once. The concentrations of particulate matter, PAHs and carbon monoxide in the chamber were regularly monitored. The particulate matter was collected on a glass fiber filter using a low volume air sampler. The amount of particulate matter was measured by weighing the filter before and after the collection of particulate matter. The PAHs in the particulate matter were analyzed using a high performance liquid chromatograph equipped with a fluorescence spectrophtometer. Concentration of carbon monoxide was measured using detector tubes.

As the increase of AHH activity was observed after 4 hrs of the exposure, rats used in the single exposure tests were sacrificed after 6 hrs, and those used in the consecutive exposure tests were sacrificed 16 hrs after the last treatment. The liver, kidney and lung of the rats were removed, weighed and homogenized in 5 vols. of ice-cold 50 mM Tris-HCI buffer(pH 7.5) containing 1.15% KCI using a Potter-Elvehjem teflon homogenizer. The homogenates were centrifuged for 20 min at 9000 x g, and then the supernatants were stored at -80°C until the enzymatic assays were performed. AHH activity in the tissue preparation was measured using a modification of the procedure of Nebert et al. (1968). The reaction for assay of AHH was carried out for 20 min at 37°C in a total volume of 1 mL. The assay contained 0.1 mL of homogenate(0.3-1.0 mg protein) and 0.9 mL of reaction mixture, which contained 50μmoles Tris-HCI buffer(pH 7.5), 0.36 μmole NADPH and 3 μmoles $MgCl_2$. Just prior to incubation, 10 μL of acetone containing 80 nmoles of benzo[a]pyrene was added to the mixture. The reaction was stopped by adding 1 mL of cold acetone. Benzo[a]pyrene and its metabolites were extracted twice with 3 mL of ethyl acetate. The 3 mL of the organic phase were evaporated to dryness under a nitrogen stream, and the residue was dissolved in 0.5 mL of methanol. The AHH activity was determined by a fluorometric measurement of 3-hydroxybenzo[a]pyrene(3-OH-BaP) using high performance liquid chromatography. The liquid chromatography was as follows: column; TSKgel ODS 80Tm(TOSOH, Japan,150 x 4 mm, id), mobile phase; methanol:water(85:15,v/v), flow rate; 0.8 mL/min, excitation wavelength; 380 nm, emission wavelength; 440 nm. The specific activity of AHH is expressed as ng·3-OH-BaP/min/mg·protein at 37°C. The protein content of the homogenates was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

RESULTS AND DISCUSSION

After the single exposure, the activities of hepatic, renal and pulmonary AHH were determined as a function of time. As shown in Fig. 1, there was a 4 hr time lag between the smoke inhalation and the enzyme induction onset. The activities decreased during 0-2 hrs after treatment in all the tissues. Thereafter, the activities increased to maximal extent after 16

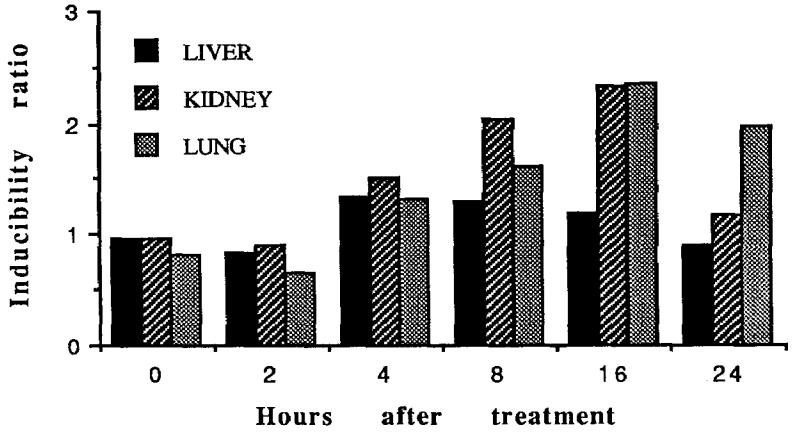

Figure 1. Effect of a single exposure to cigarette smoke on AHH in the rat liver, kidney and lung.
Rats were exposed to cigarette smoke of 15 puffs for 10 min and were subsequently sacrificed to determine AHH activity at the indicated timepoint.
Each value represents the mean of 3 animals.

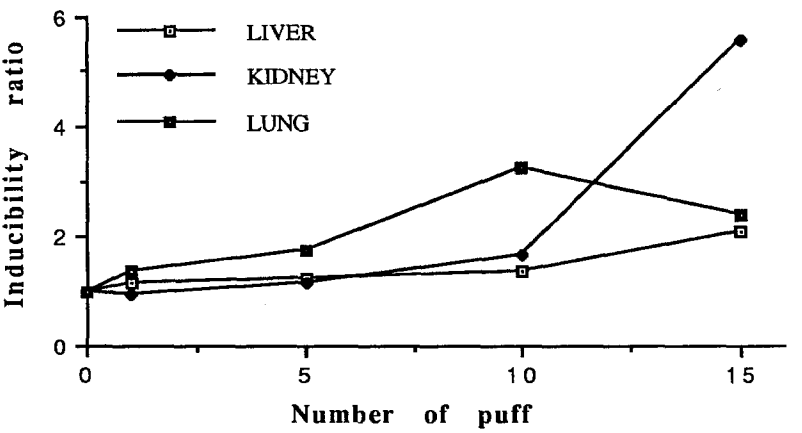

Figure 2. Dose-response relationship between inducibility ratio of AHH activity and the number of puff.
Rats were exposed to cigarette smoke of 1, 5, 10 or 15 puffs for 10 min, and were sacrificed after 6 hrs.
Each value represents the mean of 3 animals.

hrs in the kidney and lung, but after 4 hrs in the liver. In both the liver and kidney, the activities were reduced to the control values after 24 hrs, whereas in the lung the activity remained twice that of the control value. The hepatic, renal and pulmonary AHH activities reached maximum values at about 16 hrs after treatment, which is in agreement with previous report(Van Cantfort et al. 1977).

The dose-response relationship between AHH activity and the number of puffs of cigarette smoke(concentration of cigarette smoke) was determined. As shown in Fig. 2, the hepatic, renal and pulmonary AHH were induced by cigarette smoke. With an increase in the number of puffs, the AHH activities tended to increase in all the tissues. Inducibility ratios of the pulmonary AHH were higher than those of the hepatic and renal AHH under the low concentration of cigarette smoke up to 10 puffs. Therefore, the pulmonary AHH activity is more sensitive to cigarette smoke inhalation than those of the liver and kidney. Under the condition of 10 puffs, the pulmonary AHH activity reached a maximum value corresponding to 3.3 times that of the control value, while under the condition of 15 puffs, the hepatic and renal AHH activities reached maximum values corresponding to 2.1, 5.6 times of the control values, respectively.

AHH activity was evaluated after the consecutive exposure of cigarette smoke. Rats were exposed to cigarette smoke of 1, 3 or 5 cigarettes/hr, 8 hrs/day, for 5 consecutive days, and sacrificed 16 hrs after the last treatment. Table 1 shows the concentrations of particulate matter, PAHs and carbon monoxide in the exposure chamber during the consecutive exposures. As shown in table 2, the hepatic, renal and pulmonary AHH activities were affected differently when the concentrations of cigarette smoke were changed. The hepatic AHH activity increased slightly under the condition of a cigarette/hr. However, the hepatic activities were much inhibited by 3 or more cigarettes/hr. Inducibility ratios of the hepatic activity decreased with an increase in the number of cigarettes. The renal AHH activities were not affected by 1 or 3 cigarettes/hr, but the activity increased to 2.8 times the control value by 5 cigarettes/hr. The pulmonary AHH activities were greatly increased by 1, 3 or 5 cigarettes/hr. Inducibility ratios of the pulmonary AHH activity decreased with an increase in the

Table 1. The concentrations of particulate matter, PAHs and carbon monoxide in the exposure chamber during the consecutive exposures

Condition		Cigarettes/hr		
		1	3	5
Particulate matter	(mg/m^3)	25	48	71
C O	(ppm)	5-200	100-250	200-350
B(a)P	(ng/m^3)	198	458	875
B(k)F	(ng/m^3)	37	100	181
B(ghi)P	(ng/m^3)	205	433	775

B(a)P; benzo(a)pyrene, B(k)F; benzo(k)fluoranthene; B(ghi)P; benzo(ghi)perylene. Range or mean value during a hour are represented.

Table 2. Effect of consecutive exposure to cigarette smoke on AHH activity in the rat liver, kidney and lung

No. of	AHH activity(ng. 3-OHBaP/min/mg.protein)		
ciga./hr	Liver (IND)	Kidney (IND)	Lung (IND)
Control	33.3±3.8(1.00)	1.99±0.45(1.00)	0.86±0.25(1.00)
1	37.6±3.0(1.13)[a]	1.96±0.32(0.98)	6.40±0.59(7.44)[b]
3	28.2±5.1(0.85)[a]	1.94±0.14(0.97)	4.74±0.79(5.51)[b]
5	21.9±4.5(0.66)[b]	5.60±0.92(2.81)[b]	3.29±0.51(3.83)[b]
5(4hrs)	20.7±4.3(0.62)[b]	4.66±0.30(2.34)[b]	1.95±0.30(2.27)[b]

Rats were exposed to cigarette smoke of 1, 3 or 5 cigarette/hr,8 hrs/day, for 5 consecutive days, and to cigarette smoke of 5 cigarettes/hr,for 4 hrs only once. Rats were sacrificed 16 hrs after treatment. Each value represents the mean±SD(n=5). IND: Inducibility ratio.
[a] $p<0.05$ compared with control. [b] $p<0.01$ compared with control.

number of cigarettes. Maximal inducibility ratio was 7.4 under the condition of a cigarette/hr. One group of rats was exposed to cigarette smoke of 5 cigarettes/hr for 4 hrs only once. The results are also showed in Table 2. The hepatic AHH activity was inhibited and similar to that of the 5 cigarettes/hr, 8 hrs/day, for 5 consecutive days. The renal and pulmonary AHH activities increased and their inducibility ratios were 2.3, 2.4, respectively.

Bilimoria et al. (1980) and Graziano et al. (1984) have shown that the hepatic AHH activity of rats was not induced by cigarette smoke, while Kushinsky et al.(1976) and Raunio et al.(1983) have observed some increase of hepatic AHH activity in rats. In the present study, the results indicate that AHH was induced by cigarette smoke in liver, kidney, and lung. However, the hepatic AHH was inhibited by the higher concentration of cigarette smoke under both the single and consecutive exposure(Table 2). Raunio et al.(1983) detected elevations in hepatic AHH activity in rats after a single exposure to cigarette smoke and also after 3 days of exposure. However, when exposure was continued through 10 days, the hepatic AHH activity returned to a control value. As the concentrations of cigarette smoke in our experiment were higher than those reported by Raunio et al.(1983), the inhibition of the hepatic AHH activity in our experiment might have occurred. The renal AHH was induced under the condition of 5 cigarettes/hr, but not modified by 3 or less cigarettes/hr. This observation in the kidney may result in a short half-life of renal AHH activity(Van Cantfort et al. 1977). The pulmonary AHH was greatly induced under the condition of 1, 3 or 5 cigarettes/hr. The inducibility ratios in the pulmonary AHH decreased with an increase in the number of cigarettes. The rates of decrease of hepatic and pulmonary inducibility ratios were very similar. This observation may be the cause of the presence of a common inhibiting factor(s).

It is concluded, therefore, that the hepatic, renal and pulmonary AHH in rats were induced by cigarette smoke, and that there was a dose-response relationship between inhalation of cigarette smoke and AHH activity after the single exposure. The results in the present study also indicated that hepatic AHH activity was inhibited under the condition of high concentration of cigarette smoke.

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