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INDUCTION OF ARYL HYDROCARBON HYDROXYLASE BY 3-METHYLCHOLANTHRENE IN LIVER, LUNG AND KIDNEY OF GONADECTOMIZED AND SHAM-OPERATED WISTAR RATS*

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Abstract—Detailed dose–response curves have been obtained for the induction of aryl hydrocarbon hydroxylase (AHH) in liver, lung and kidney by intraperitoneally administered 3-methylcholanthrene (3-MC) in Wistar rats. The effects of gonadectomy also were studied. Lung was the tissue most sensitive to induction, followed by liver, and then kidney in all cases. Although liver exhibited the greatest overall activity, the per cent increase over control AHH levels was much higher in the two extrahepatic tissues. Gonadectomy did not affect either control or induced AHH activity in any of the three organs in the female, or in the male lung. However, castration of the males decreased control liver enzyme levels and increased these levels in kidney. The AHH levels reached after maximal 3-MC induction were the same in castrated and sham-operated male rat livers. A different pattern was seen in male kidney enzyme levels where significantly increased maximal induction was observed in castrated animals.

Induction of hepatic drug-metabolizing enzymes has been a topic of research interest for many years. Recently extrahepatic enzyme induction has been receiving a great deal of attention. The assay of benzo(a)pyrene hydroxylase activity has been a convenient tool with which to follow the induction of the cytochrome P_1-450 mixed function oxidase system. It has been found that the activity of benzo(a)pyrene hydroxylase can be correlated directly with the formation of reactive and, therefore, potentially toxic metabolites, as measured by irreversible binding to lung macromolecules [1]. The presence of benzo(a)-pyrene hydroxylase in rat lung, kidney, testis, thyroid and adrenal glands has been shown histochemically [2]. In an early paper [3], induction of this enzyme by 3-methylcholanthrene (3-MC) in lung, kidney and intestines of male rats also was demonstrated. Some examples of more recent induction studies done using other species include that of Law *et al.* [4] (guinea pig lung), Burke and Prough [5] (hamster lung), and Seifried *et al.* [6] (mouse lung). In most cases the route of administration of the inducing agent (usually 3-MC) has been intraperitoneal. However, only a few researchers have measured induction of a single enzyme over a complete dose range in extrahepatic tissues [7]. Rather, the majority have looked at a variety of enzymes induced by many drugs [8, 9]. In order to evaluate adequately the relative changes in tissue enzyme levels brought about by inducing agents, complete dose–response curves are necessary. Otherwise, comparisons are made based on a response which may be maximal in one tissue, but much less than maximal in another. Therefore, the following experiments were carried out. The levels of aryl hydrocarbon hydroxylase (AHH) were measured by following the hydroxylation of benzo(a)pyrene (i.e. benzpyrene hydroxylase), with and without 3-MC pretreatment. The assays were carried out in liver, lung and kidney tissue of both male and female rats, since sex-related differences in drug-metabolizing enzymes are well-known [10].

MATERIALS AND METHODS

Chemicals. 3-Methylcholanthrene was supplied by Eastman Organic Chemical Co., Rochester, N.Y. All other chemicals and biochemicals were obtained from Sigma Chemical Co., St. Louis, MO. The benzo(a)pyrene (BP) was recrystallized from benzene for further purification.

Animals. Wistar rats were obtained from Canadian Breeding Farms Ltd., La Prairie, Quebec, where they were castrated, ovariectomized or sham-operated at 3–4 weeks of age. They were raised under controlled conditions (22°, lights on 6:00 a.m. to 8:00 p.m.) on corncob bedding (Lobund grade, Paxton Processing Ltd., Paxton, IL). Locally supplied Wistar rats used in the second set of experiments were obtained at U.B.C. Purina lab chow and water were available at all times, *ad lib.* The induction experiments were carried out on 160–260 g females, and 260–370 g males. Control and experimental groups were matched by age and weight. Corn oil or 3-MC (as a solution in corn oil) was injected intraperitoneally (i.p.) (maximum volume, 0.5 ml) 48 and 24 hr prior to sacrifice.

Enzyme preparation. Animals were stunned by a blow on the head, killed by decapitation and bled. The lungs, liver and kidneys were perfused with 1.15% KCl, removed, and placed in ice-cold 1.15% KCl.

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All procedures were subsequently carried out at 4°. The tissues were blotted, minced and homogenized 1:4 (w/v) in KCl for liver or 1:2 (w/v) in KCl for lung and kidney (1 min 15 sec for liver, 1 min 30 sec for kidney, and 4 min for lung). The homogenates were centrifuged for 10 min at 10,000 *g*; the lung and kidney supernatant fractions were used in the enzyme assay. The liver supernatant fraction was centrifuged at 100,000 *g* for 1 hr; the pellets were washed with 0.2 M phosphate buffer, pH 7.2, and resuspended in this buffer.

Enzyme assay. The requirements for linearity and optimal benzpyrene hydroxylase activity (AHH) in each of the tissues were determined using a modification of the method of Van Cantfort and Rondia [11]. The final volume of the incubation mixture was 1 ml and contained for liver: 0.36 μmole each of NADH and NADPH, 3 μmoles $MgCl_2$, 0.6 mg bovine serum albumin, 150 μl enzyme preparation, 80 nmoles BP in 40 μl acetone and 25 μmoles Tris buffer, pH 7.4; for lung and kidney: 1.08 μmoles each of NADH and NADPH, 9 μmoles $MgCl_2$, 1.8 mg bovine serum albumin, 450 μl enzyme preparation, 80 nmoles BP in 40 μl acetone and 25 μmoles Tris buffer, pH 7.4. The incubation was performed at 37° for 2.5 min for liver, 5 min for kidney and 15 min for lung. High protein concentrations were employed in order to overcome the low enzyme activity in control kidney tissue preparations. Longer incubation time periods than those used for kidney resulted in non-linearity, a problem also noted by others (see Ref. 7, Fig. 3A). It was verified by control experiments that the 100,000 *g* supernatant fraction did not change AHH activity. Also, hepatic 10,000 *g* supernatant and 100,000 *g* pellet fractions had similar levels of activity per mg of protein. In order that the enzymes from all three tissues could be assayed within a reasonable time period and still allow direct comparison between the present results and those of previous investigators, the 10,000 *g* supernatant fraction was employed for both extrahepatic tissues. The formation of 3-hydroxy-benzpyrene was measured by the method of Nebert and Gelboin [12]; proteins were analyzed by the method of Sutherland *et al.* [13] as modified by Robson *et al.* [14].

RESULTS

Induction of AHH by 3-MC in female rats. Ovariectomized or sham-operated females were treated with a range of doses of 3-MC (0–40 mg/kg). The AHH activities in liver, lung and kidney were measured

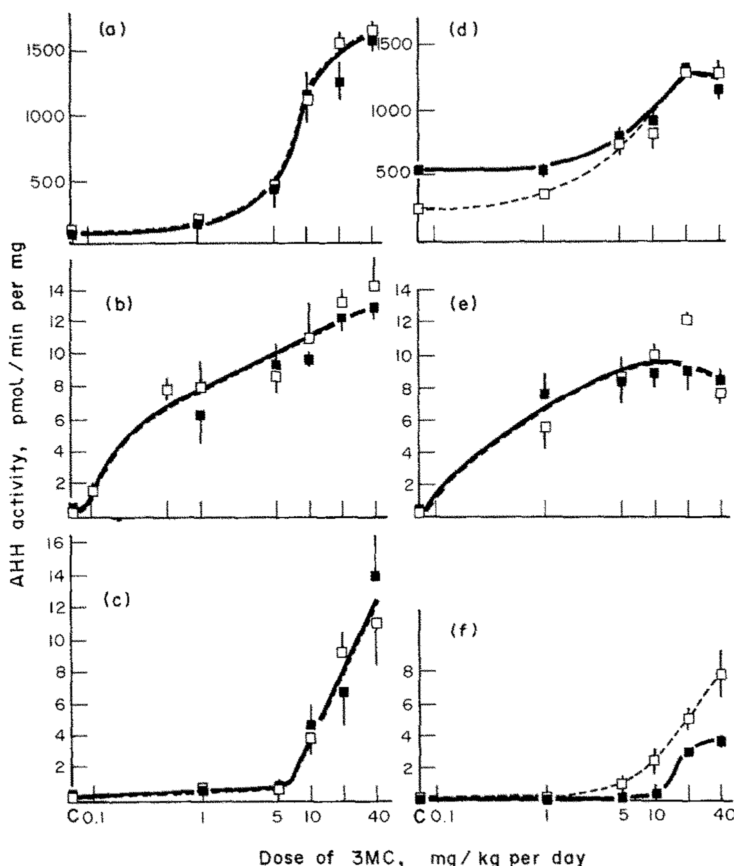

Fig. 1. Effect of 3-methylcholanthrene (3-MC) on aryl hydrocarbon hydroxylase (AHH) activity in liver, lung and kidney of sham-operated and ovariectomized female rats (panels a, b and c) or sham-operated and castrated male rats (panels d, e and f). Animals were injected intraperitoneally with 3-MC 48 and 24 hr prior to killing; AHH activity was determined as described in Materials and Methods. Each point represents the mean of four female rats ± S.E.M. or of eight male rats ± S.E.M., except at 20 mg/kg where $n = 4$. Key: C = control enzyme levels; ■ = sham; □ = gonadectomized; and the asterisk = statistically different, $P < 0.05$.

and dose–response curves were obtained (Fig. 1, panels a, b and c). There was no significant difference in the AHH activities of sham and ovariectomized females in any of the tissues at the control levels or after 3-MC treatment. Gurtoo and Parker [15], studying non-induced enzyme levels, did not notice any difference between normal and ovariectomized rat kidney or liver. Guenthner and Mannering [16] have shown recently that even the large elevations in levels of female sex hormones seen in pregnancy did not increase hepatic AHH activity in rats.

As expected, the degree of response differed from tissue to tissue at any one dose. A 5 mg/kg dose of 3-MC produced a significant increase in AHH activity in the lung and liver, whereas the AHH activity in the kidney was still at control levels (Fig. 1). At very low 3-MC doses, the enzyme levels in the lung were elevated significantly. Increased AHH activity could be detected at 0.1 mg/kg ($P < 0.02$) and began to level off at 10 mg/kg. In contrast, apparent AHH induction in the kidney was not seen until 10 mg/kg of 3-MC was injected, from which point it continued to rise linearly. At 40 mg/kg of 3-MC, the kidney enzyme level had reached that of the lung.

Induction of AHH by 3-MC in male rats. Castrated or sham-operated males were injected with the same range of doses of 3-MC as the females. The dose-response curves obtained are given in Fig. 1 (panels d, e and f). Differences in control levels of liver AHH activity between sham and castrated males were clearly evident, as expected (see Refs. 15, 17 and 18: using sexually mature and immature animals). Castration appeared to have a feminizing effect, lowering the AHH activity in the liver toward that found in females. However, castration produced no change in AHH activity in the lung or kidney of control animals. The results in kidney are in accord with those of Gurtoo and Parker [15], who also noted no change in AHH activity between sham and castrated males.

As the dose of 3-MC increases, the differences in liver AHH activity between sham and castrated rats disappear, until at 5 mg/kg there was no significant difference between the two groups. Conversely, in the kidney, differences became evident at 5 mg/kg and were greatly increased at 40 mg/kg. At this dose AHH activity in castrated males was twice as high as that in sham-operated males, an exact reversal of the effect castration had upon the liver. In the lung, however, there were no significant differences in AHH activity between castrated and sham-operated groups. As was the case in the females, lung AHH activity was induced rapidly and reached a maximum level at the comparatively low dose of 5 mg/kg. The maximum level of AHH activity in the lung was identical to the level reached in the kidneys of castrated male rats (40 mg/kg).

Comparison of male and female AHH activity. When the control levels of AHH activity in sham-operated males and females were compared in each tissue, significant sex-related differences were evident. Control AHH activity of female tissue was higher than that of male lung (0.67 ± 0.2 vs 0.25 ± 0.08 pmole/min/mg) and kidney (0.22 ± 0.06 vs less than 0.09, i.e. the lower limit of detection, in pmole/min/mg). However, the reverse was true in liver (606 ± 40 vs 105 ± 7.1 pmoles/min/mg, males vs females respectively). This is in partial agreement with Gurtoo and Parker [15], and Chhabra and Fouts [19] who also noted higher levels in female rat kidney and lower levels in female liver. However, neither study detected any sex difference in lung AHH activity.

When non-induced gonadectomized animals were compared for sex-related differences, similar results were obtained, with one exception. In the liver of castrated males, the control enzyme levels approached female levels. This is also in agreement with other workers [15].

For both sham-operated and gonadectomized animals, the maximum induced AHH level reached in the liver and the lung appeared to be significantly higher in the females than in the males. However, the differences may be biologically trivial. Sex-related differences in the kidney were also evident, but exhibited an unusual pattern. The same maximum activity occurred in female sham and ovariectomized rats and in castrated males. However, the sham-operated males exhibited a significantly lower maximal AHH activity (only 46 per cent of the castrated males). This would indicate that castration again had a feminizing effect, but in this instance it resulted in increased activity (not a decrease as in non-induced male livers). The ED_{50} values for induction by 3-MC calculated from the respective dose–response curves are as follows: male liver, sham-operated: 2.7 mg/kg; castrated: 4.5 mg/kg; female liver: 7.2 mg/kg; male lung: 0.38 mg/kg; female lung: 0.5 mg/kg; male kidney: 15.5 mg/kg; and female kidney: 14.5 mg/kg.

Induction of AHH by 3-MC—Vancouver subline of Wistar rat. To determine if the patterns of induction in the three tissues could be duplicated in another rat subline, male and female Wistar rats (not gonadectomized) were obtained from local sources and injected i.p. with 3-MC (0–40 mg/kg for females and 0–60 mg/kg for males). Liver, lung and kidney were assayed for AHH activity as in the previous set of experiments (Table 1). Induction was apparent in all three tissues from both sexes. A general pattern similar to that found in the previous study was seen, in that lung AHH was induced at the lowest dose used, higher doses were necessary for liver, and still higher doses for kidney. Again the maximum level of AHH activity in the lung was reached at a very low dose (1 mg/kg in the males, 5 mg/kg in the females) even though the route of administration was intraperitoneal.

In order to clarify better the dose necessary for maximal induction in the kidney, 60 mg/kg of 3-MC was administered to the male rats. Even at this dose, the enzyme activity was seen to continue rising linearly.

In this subline, the only obvious sex difference noted was the lower AHH activity present in non-induced female liver as compared to that of the corresponding males. Otherwise, the dose–response curves for the two sexes did not differ significantly.

DISCUSSION

When dose–response curves are drawn for AHH induction in each tissue, comparisons of responsiveness between organs can be made easily. In the present study, rats received doses of 3-MC intraperitoneally;

Table 1. Effect of 3-methylcholanthrene (3-MC) on aryl hydrocarbon hydroxylase (AHH) activity in liver, lung and kidney of local subline of Wistar rats.*

Sex	Dose (mg/kg/day)	AHH activity (p moles/min/mg)		
		Liver	Lung	Kidney
Male	0	477 ± 36	0.72 ± 0.13	†
	0.1		2.7 ± 0.9	
	0.3		6.1 ± 0.1	
	1	460 ± 16	14.7 ± 1.5	0.09 ± 0.06
	5	895 ± 57	12.5 ± 0.2	0.47 ± 0.19
	10	1476 ± 68	12.3 ± 1.3	3.94 ± 0.76
	20	1650 ± 101	12.9 ± 1.8	8.00 ± 3.3
	40	1481 ± 86	11.9 ± 1.4	15.1 ± 1.0
	60	1540 ± 24	13.1 ± 0.5	22.1 ± 2.3
Female	0	67 ± 3	0.55 ± 0.06	†
	5	856 ± 93	12.2 ± 0.9	3.26 ± 0.20
	10	1407 ± 71	11.1 ± 1.5	5.33 ± 0.92
	20	1763 ± 61	9.6 ± 1.5	5.33 ± 2.2
	40	1513 ± 107	8.4 ± 0.9	17.8 ± 5.4

* Animals were injected intraperitoneally with 3-MC 48 and 24 hr prior to being killed. AHH activity was determined as described in Materials and Methods; $n = 4$. Values are expressed as the mean ± S.E.M.
† Lower limit of detection.

induction was evident in the lung at the lowest dose, followed by liver, then kidney. Previous investigators did not attempt to determine the minimum dose level at which induction could be detected in each organ [8, 9].

Our results can be compared to those of Van Cantfort and Gielen [20, 21], in which AHH levels in the liver, lung and kidney were measured in male Sprague–Dawley rats after inhalation of increasing concentrations of cigarette smoke. In these particular studies, dose–response curves similar to those of the present study were obtained for lung and kidney (i.e. rapid induction in lung, slower onset in kidney); however, there was no detectable induction in the liver. These authors explained their results by taking into account the route of administration and the pattern of blood flow. The rapid onset and the high AHH levels in the lung would be expected, since it was the first organ exposed. The fact that the lung did not detoxify all of the compound and that enough active compound reached other organs to produce induction has interesting implications. However, a different conclusion may be reached from data in the present study, in which the route of administration was intraperitoneal. Even though the liver was exposed first and in highest concentrations to 3-MC, the lung was the organ most sensitive to induction. In addition, it is interesting that the lung was induced 33-fold whereas the liver was induced only 2.2-fold (sham males). For these reasons, local effects of toxic metabolites formed in such areas as the lung are likely to be of importance, i.e. these tissues may exhibit increased susceptibility to tumor induction. Since we did not determine the chemical form of the 3-MC in the various tissues, it is possible that different metabolites may be acting as inducers in different tissues, as suggested by Van Cantfort and Gielen [21].

Two different sublines of Wistar rats were used in this study, and although the AHH levels reached in the organs differed between the two groups, the general patterns and trends were similar. One notable difference, however, occurred in the kidney where the maximal induced enzyme levels were similar in both sexes, whereas in the first group they had differed significantly. Differences in drug metabolism among species have been reported many times before [22–26]. Although the genetic background of mice has been well characterized, the same is not true of rats. Sublines may differ with respect to drug metabolism, as suggested in the present work.

It is well known that sex hormones affect drug-metabolizing enzymes [26–28]. In the liver of male rats, a number of substrates are metabolized more rapidly than in female rats [18, 22]. In addition, the adrenals and pituitary play a role in induction [28–30]. Recently, Kramer *et al.* [31] have shown induction of androgen-dependent hepatic enzymes by luteinizing hormone and follicle stimulating hormone. The mechanisms responsible for induction by sex hormones (e.g. testosterone) and 3-MC are different [10]. The magnitude, direction and sensitivity of the response also vary between the two inducing agents and the various tissues. From the present studies we cannot conclude what part the adrenals or pituitary play in the induction of AHH. However, although sex differences appear relatively small, they may be very important toxicologically [32]. The present data, along with that of Gurtoo and Parker [26], indicate that sex differences vary with strain, tissue, and level of induction.

It has been shown that sex-specific differences in the site of neoplasms produced by 3-MC occur in rats [33]. It is also known that, although 3-MC is found in high concentrations in rodent liver, lung and kidney [34], the major site of carcinogenesis is lung [34–36]. 3-MC is not carcinogenic in liver [34, 37].

Since this polycyclic hydrocarbon is bound to specific lung components [38] but no binding occurs to liver DNA [37], we are currently determining the relationship between binding of 3-MC to nucleic acids and changes in DNA damage at various points on the AHH dose–response curves in the three tissues studied.

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