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EFFECTS OF THE 1-KETO AND 1-HYDROXY DERIVATIVES OF 3-METHYLCHOLANTHRENE UPON LIVER DRUG METABOLIZING ENZYME ACTIVITY*

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Abstract—The effects of 2 oxidized derivatives of 3-methylcholanthrene upon the drug metabolizing enzyme system in liver were determined. 3-Methylcholanthrene-1-one was 60 per cent as effective as the parent compound in stimulating the activity of benzpyrene hydroxylase in liver after intraperitoneal administration, while the 1-hydroxy derivative was ineffective in this regard. The administration of 3-methylcholanthrene to rats resulted in a 3-fold increase in the ability of liver microsomes to metabolize zoxazolamine, while the administration of the 1-keto and the 1-hydroxy derivatives produced a stimulatory activity of only 2- and 1·7-fold respectively. The rates of zoxazolamine metabolism were correlated with the duration of zoxazolamine-induced paralysis observed after administration of the methylcholanthrene derivatives to rats.

3-METHYLCHOLANTHRENE is a polycyclic hydrocarbon which possesses potent pharmacologic properties. Under suitable conditions, administration of the polycyclic hydrocarbon is followed by the appearance of a variety of widely disseminated neoplasias in a wide spectrum of animal species. However, the carcinogenic influence of 3-methylcholanthrene does not extend to the liver of the adult rat.

The polycyclic hydrocarbon elicits another pharmacologic activity—its intraperitoneal administration leads to the 'induction' of some of the enzymes which function in biotransformation reactions and which are located in the endoplasmic reticulum of liver.[1–3] This elevation in enzyme activity may result in the inhibition of liver carcinogenesis as induced by such agents as 3′-methyl-4-dimethylaminoazobenzene,[4–6] 2-fluorenylacetamide,[6–8] and 7,12-dimethylbenz(α)-anthracene.[9–11]

The different effects of 3-methylcholanthrene may in part be because of different metabolites of the polycyclic hydrocarbons. In this regard, Sims[12] has demonstrated that several of the oxidized derivatives of 3-methylcholanthrene are carcinogenic when applied subcutaneously. However, it is not known whether the administration of these derivatives may result in differential effects in stimulating the activity of the drug-metabolizing enzyme system. Accordingly, the effects of administration of the 1-hydroxy and 1-keto-3-methylcholanthrene upon the activity of microsomal benzpyrene hydroxylase and upon the metabolism of zoxazolamine have been compared with the stimulatory effect of the parent compound. The results indicate that 3-methylcholanthrene-1-one is an effective 'inducer' and the 1-hydroxy much less active in this regard.

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MATERIALS AND METHODS

Polycyclic hydrocarbons. 3-Methylcholanthrene was obtained from Eastman Organic Chemicals and was kept in a dark bottle away from light to avoid photodecomposition. The 1-keto compound was prepared by dichromate oxidation of 3-methylcholanthrene.[13] The 1-hydroxy derivative of the polycyclic hydrocarbon was prepared as described by Sims[14] by reduction of 3-methylcholanthrene-1-one with lithium aluminium hydride. The ultraviolet absorption spectra and melting points agreed with those of Sims.[14]

The purity of the derivatives was assessed by melting point and by thin-layer chromatography on silica gel-coated plastic foils (Eastman Organic Chemicals) using benzene:ethanol (100:1) and benzene:ethanol (100:3) as developing solvents. The parent polycyclic hydrocarbon, 3-methylcholanthrene, travelled with an R_f of 0·95 in both solvents; the 1-keto derivative had an R_f of 0·57 and 0·84 respectively; the 1-hydroxy had an R_f of 0·36 and 0·59 respectively. The authors are grateful to Dr. Peter Sims of the Chester Beatty Research Institute who provided authentic samples of the 1-hydroxy and 1-ketone derivatives of 3-methylcholanthrene for comparative purposes.

Animals. Male Cheek–Jones rats, 50–80 g in weight, were used in all the experiments. They were allowed free access to both Purina lab. chow and water. 3-Methylcholanthrene and derivatives were dissolved in corn oil at 2 mg/ml and were given intraperitoneally at approximately 20 mg/kg body weight. Occasionally, the 1-hydroxy derivative was dissolved in propylene glycol. Control rats received either corn oil or propylene glycol intraperitoneally

Zoxazolamine solutions were prepared as described by Conney *et al.*[15] by adding 300 mg of the drug to 3·6 ml of 1 N HCl. The solution was diluted with 0·9% saline to 15 ml and filtered. Aliquots of the filtrate were injected intraperitoneally into the rats at a dose of 100 mg/kg. Control rats received 0·9% saline. The duration of zoxazolamine paralysis was determined by recording the time when the rats regained their righting reflex. The toxicity of strychnine phosphate was determined after the injection of the drug at 3·5 mg/kg intraperitoneally to 80–100 g male rats.

Enzyme assays. The rats were killed by exsanguination after administration of ether anesthesia. The livers were removed, washed in cold 0·25 M sucrose, and a 20% homogenate was prepared in the latter by grinding in a Potter–Elvehjem homogenizer. The homogenate was centrifuged at 9000 *g* for 10 min in a Sorvall RC-2 refrigerated centrifuge, and the supernatant fraction was collected and employed in the enzyme assays.

The metabolism *in vitro* of zoxazolamine was measured as described by Conney *et al.*,[15] using a spectrophotometric method.[16] The activity of benzpyrene hydroxylase in the 9000 *g* rat supernatant fraction was estimated by a modified Wattenberg method,[17] using the incubation mixture described by Kuntzman *et al.*[18] and measuring the fluorescence of the hydroxylated derivatives of benzpyrene in an Aminco–Bowman spectrophotofluorometer with the activation and emission wavelengths set at 396 and 522 nm respectively. The specific activity of benzpyrene hydroxylase has been expressed as the relative intensity (RI) of fluorescence due to the hydroxylated derivatives per milligram protein per 10 min assay.

The protein concentration of aliquots of the 9000 *g* preparations was determined by the method of Lowry *et al.*[19] with bovine serum albumin as the reference standard.

RESULTS

The intraperitoneal injection of 3-methylcholanthrene or its 1-keto derivative is attended by a profound elevation in the activity of liver benzpyrene hydroxylase (Fig. 1) which reached a maximum at 24 hr after administration. At this time, the increase in activity after injection of the parent polycyclic hydrocarbon and its derivative amounted to 12- and 7-fold respectively. By 72 hr after administration of the 3-methylcholanthrene-1-one, the benzpyrene hydroxylase activity had returned to within the normal range, whereas at this time after the injection of 3-methylcholanthrene, enzyme activity was still elevated, i.e. by approximately 7-fold. The 1-hydroxy derivative of 3-methylcholanthrene, in contrast, was not an effective "inducing" agent of benzpyrene hydroxylase activity.

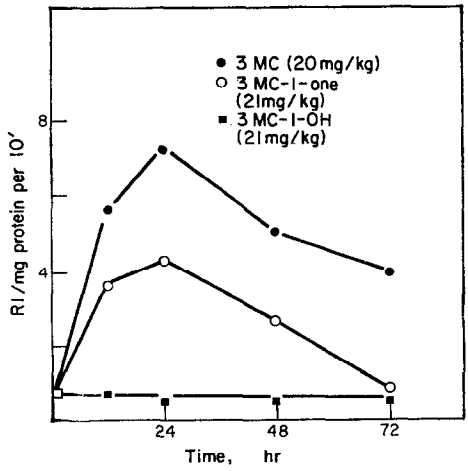

FIG. 1. Effects *in vivo* of 3-methylcholanthrene (3MC) and derivatives upon benzpyrene hydroxylase activity. Male rats, 50–80 g, were injected intraperitoneally with 3MC, (20 mg/kg), 3MC-1-one (21 mg/kg), or 3MC-1-OH (21 mg/kg), and were sacrificed periodically thereafter. The benzpyrene hydroxylase activity of 9000 *g* liver fractions was determined and is expressed as relative intensity of fluorescence (RI) per milligram of protein per 10 min. The values presented in the figure represent the averages of at least 4 determinations.

The effect of dose of the polycyclic hydrocarbon upon the activity of benzpyrene hydroxylase in liver is indicated in Table 1. Maximum elevation of enzyme activity as achieved at 20 and 21 mg/kg, of 3-methylcholanthrene and its 1-keto derivative respectively. The 1-hydroxy derivative at 28 mg/kg was still not effective in elevating benzpyrene hydroxylase activity.

Microsomal metabolism of zoxazolamine was also influenced by administration of the polycyclic hydrocarbons. These data are presented in Table 2. 3-Methylcholanthrene, its 1-keto and 1-hydroxy derivatives, were effective stimulators of the microsomal metabolism of zoxazolamine. Administration of the parent polycyclic hydrocarbon was attended by an increase of approximately 3-fold in the metabolism of zoxazolamine. The injection of the 1-keto and 1-hydroxy derivatives produced elevations of 2- and 1·7-fold respectively. The effect of these agents was also followed by observing the

TABLE 1. EFFECTS *IN VIVO* OF 3-METHYLCHOLANTHRENE AND DERIVATIVES UPON BENZPYRENE HYDROXYLASE ACTIVITY*

Treatment and dose	Benzpyrene hydroxylase (RI/mg protein/10 min)
Corn oil (7)†	0·6 (0·4–0·8)‡
Propylene glycol (3)	0·5 (0·3–0·6)
3MC, 6·7 (4)	3·6 (3·0–4·9)
(mg/kg)	
20 (5)	7·0 (5·9–8·5)
30 (5)	7·1 (5·8–8·7)
3MC-1-one, 7 (4)	1·3 (0·7–1·7)
(mg/kg) 21 (6)	4·2 (2·6–5·5)
28 (4)	3·9 (2·3–4·9)
3MC-1-OH, 21 (4)	0·6 (0·3–0·8)
(mg/kg) 28 (4)	0·5 (0·3–0·9)

* Male rats, 50–80 g, were injected intraperitoneally with 3-methylcholanthrene (3MC), 3-methylcholanthrene-1-one (3MC-1-one) or 3-methylcholanthrene-1-hydroxy (3MC-1-OH) and sacrificed 24 hr later. The latter agent was given in propylene glycol, while the 3MC and 3MC-1-one were given in corn oil. Benzpyrene hydroxylase activity in liver was determined and expressed as relative intensity of fluorescence (RI) per milligram of protein per 10 min.
† Number of determinations.
‡ Range.

TABLE 2. DURATION OF ZOXAZOLAMINE-INDUCED PARALYSIS AND METABOLISM AFTER ADMINISTRATION OF 3-METHYLCHOLANTHRENE AND DERIVATIVES*

Treatment	Duration of paralysis (min)	Per cent of control	Metabolism of zoxazolamine (μmoles/g protein/hr)
Corn oil (4)†	205 ± 17‡	100	39
3MC (5)	23 ± 4	11	103
3MC-1-one (5)	70 ± 19	34	83
3MC-1-OH (5)	103 ± 17	50	68

* Male rats, 50–80 g, were injected intraperitoneally with either 3-methylcholanthrene (3MC), 20 mg/kg, 3-methylcholanthrene-1-one (3MC-1-one), 21 mg/kg, or 3-methylcholanthrene-1-hydroxy (3MC-1-OH), 21 mg/kg. The rats were given zoxazolamine, 100 mg/kg, 24 hr later and the duration of paralysis was determined. The metabolism *in vitro* of zoxazolamine was ascertained as described in the text.
† Number of determinations.
‡ Average time ± standard deviation.

duration of zoxazolamine-induced paralysis. The administration of 3-methylcholanthrene resulted in a reduction in the duration of paralysis by 9-fold; the 1-keto and 1-hydroxy derivatives reduced the duration of this pharmacologic property by 3- and 2-fold respectively.

Strychnine toxicity, on the other hand, was not altered by the administration of the polycyclic hydrocarbons. All rats convulsed and died within 2–7 min.

DISCUSSION

Sims[14] has reported that the metabolism *in vitro* of 3-methylcholanthrene by rat

liver homogenates leads to the production of a number of oxidized derivatives including the 1-keto and 1-hydroxy compounds. Sims[12] has tested the carcinogenicity of many of the metabolites of 3-methylcholanthrene by injecting these compounds subcutaneously into mice. The 1-hydroxy and 1-keto derivatives of the polycyclic hydrocarbon were both active carcinogens, producing the same number of tumors as did the parent compound.

In contrast, however, the 1-hydroxy and 1-keto derivatives are not equally effective in stimulating the activity of the microsomal drug-metabolizing enzyme system when given intraperitoneally. In this regard, the latter compound is about 60 per cent as effective as the parent polycyclic hydrocarbon while the former produced no stimulation in benzpyrene hydroxylase activity and only minimal stimulation in the metabolism of zoxazolamine.

We have previously reported that 3-methylcholanthrene is bound to protein in the liver shortly after the administration of labeled material intraperitoneally.[20] Recently, we have been able to duplicate the binding of the polycyclic hydrocarbon to protein using 9000 *g* liver supernatant fractions.[21] In addition, it was demonstrated that prior administration of unlabeled polycyclic hydrocarbon to rats resulted in the greater ability of the 9000 *g* liver supernatant preparation to bind labeled 3-methylcholanthrene.[21] In this regard, administration *in vivo* of the 1-keto derivative resulted in an enhanced ability of the supernatant protein to bind labeled 3-methylcholanthrene as well, while administration of the 1-hydroxy compound resulted only in a marginal elevation in protein binding ability.[21] The binding and induction of binding of polycyclic hydrocarbons to proteins and nucleic acids in liver preparations have also been reported by Grover and Sims,[22] and by Gelboin.[23]

The differential effects of the derivatives of 3-methylcholanthrene upon the parameters just discussed are interesting, especially in light of the report of Sims[14] that incubation of rat liver homogenates with 1-hydroxy-3-methylcholanthrene resulted in the formation of the 1-keto compound. Similarly, the incubation of rat liver homogenates with 1-keto-3-methylcholanthrene resulted in the formation of the 1-hydroxy derivative. It is possible that the 1-hydroxy-3-methylcholanthrene is not readily absorbed after intraperitoneal administration. Perhaps this is the reason for the apparent lack in stimulatory activity upon the drug metabolizing enzyme system. It is also conceivable that the 1-hydroxy derivative is either very rapidly bound to protein or is rapidly conjugated to glucuronide and eliminated.

A recent study by Dewhurst and Kitchen[24] has shown the elimination of the stimulatory activity of benzpyrene administration upon zoxazolamine metabolism by the introduction of a hydroxymethyl group. In this regard, 6-methyl benzpyrene administration resulted in a marked reduction in zoxazolamine-induced paralysis time. 6-Formyl benzpyrene was totally ineffective, while after 6-hydroxymethyl benzpyrene administration, a prolonged zoxazolamine-induced paralysis time was noted.

It is apparent that oxidation of the polycyclic hydrocarbon nucleus can alter some pharmacologic properties and not others. It would be of interest to see if different cell types exhibited varying sensitivities to the carcinogenic actions of the oxidized derivatives of 3-methylcholanthrene. Perhaps the spectrum of tumors produced after the administration of this polycyclic hydrocarbon is caused by the number and nature of the metabolites.

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