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EFFECTS OF ORGANIC SOLVENT VEHICLES ON BENZO[*a*]PYRENE METABOLISM IN RABBIT LUNG MICROSOMES

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Abstract—In order to study the metabolism of benzo[*a*]pyrene (BP), it must be dissolved in an organic solvent vehicle for delivery to the tissue. We studied the effects of five organic solvent vehicles, i.e. dimethyl sulfoxide (DMSO), acetone, methanol, ethanol, and ethyl acetate, on benzo[*a*]pyrene hydroxylase activity and the BP metabolite profile in rabbit lung microsomes. Fluorescence detection of 3- and 9-OH-BP was used to evaluate benzo[*a*]pyrene hydroxylase activity, and the BP metabolite profile was obtained by HPLC analysis. All solvent vehicles inhibited benzo[*a*]pyrene hydroxylase in a dose-dependent manner. When the smallest volume of each solvent (10 μl/ml reaction mixture) was employed, the resulting enzyme activities as related to solvent type, from highest to lowest, were DMSO ≥ methanol > ethanol ≥ acetone > ethyl acetate. HPLC analysis of BP metabolites formed in the presence of the five solvent vehicles showed that production of all metabolites was greatest when DMSO was used and that linearity of product formation was retained longer with DMSO. The metabolites produced when DMSO was used as the solvent were BP-9,10-diol, BP-4,5-diol, BP-7,8-diol, BP-1,6-quinone, BP-3,6-quinone and 3-OH-BP. A similar metabolite profile was obtained when reactions were carried out with methanol as the solvent vehicle, although the magnitude of production was less than with DMSO. When acetone was used, there were greater amounts of BP-4,5-diol and BP quinone formation and lesser amounts of 3-OH-BP formed than with DMSO or methanol. When ethanol or ethyl acetate was used as a solvent, BP-9,10-diol and 3-OH-BP were the only metabolites produced. These results indicate that all solvent vehicles studied inhibit benzo[*a*]pyrene hydroxylase from rabbit lung microsomes in a dose-dependent manner and that the magnitudes and types of metabolites formed are highly dependent upon the specific solvent used as the vehicle. The study also indicates that DMSO is probably the solvent vehicle of choice for study of BP metabolism in rabbit lung microsomes.

Benzo[*a*]pyrene (BP), a polycyclic aromatic hydrocarbon, is a ubiquitous pollutant which enters the environment primarily from the combustion of organic materials [1]. After entry into the body, BP may be metabolized by the mixed-function oxidative system (MFOs) found in various tissues. This enzyme system is most active in mammalian liver but is also found in extrahepatic tissues such as lung, skin, colon and kidney [2]. The MFOs functions in the conversion of hydrophobic substrates to water soluble products that are excreted in the bile and urine. However, during the metabolism of BP, reactive intermediates which have been identified as ultimate carcinogens and/or mutagens [3, 4] are produced. For this reason, the metabolism of BP has been studied extensively in various tissues. The study of pulmonary BP metabolism is particularly important since BP may enter the lungs bound to airborne particulates. Furthermore, the lung appears to be very susceptible to polycyclic hydrocarbon carcinogenesis [5, 6], although MFO activity has been reported to be considerably lower there than in liver [5].

In order to study the metabolism of BP by living tissues, it must be solubilized in organic solvents for delivery to the tissue [7]. Various solvent vehicles have been utilized to solubilize BP in procedures developed to evaluate its metabolism [8–12]. In most studies of BP metabolism in lung, either acetone [13–17] or methanol [18–22] is the solvent vehicle commonly used. We have found, however, that in rabbit lung microsomes BP is metabolized to a greater extent when dimethyl sulfoxide (DMSO) is used as the solvent vehicle than when either acetone or methanol is employed. These findings suggested that the choice of solvent vehicle may substantially influence the rate of pulmonary BP metabolism. Therefore, we studied the effects of five organic solvent vehicles, i.e. DMSO, acetone, methanol, ethanol and ethyl acetate, on BP metabolism in rabbit lung microsomes. The specific objectives were to determine the effects of the five solvent vehicles on benzo[*a*]pyrene hydroxylase activity and on the benzo[*a*]pyrene metabolite profile. A preliminary report of this work has appeared previously [23].

MATERIALS AND METHODS

Male New Zealand white rabbits (2–4 kg), supplied by Green Meadows Rabbit Farm, Murrysville,

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PA, were used throughout the study. The rabbits were killed by injection of air into the marginal ear vein, and the lungs and heart were immediately removed *en bloc*. The heart, adipose tissue, blood vessels, trachea and connective tissue were removed, and the lung tissue was blotted dry and weighed. The lungs were minced with a McIlwain Tissue Chopper (Mickle Engineering Co., Gomshall, Surrey, U.K.) set at a slice thickness of 5 mm, and the tissue mince was suspended in ice-cold 0.1 M phosphate buffer (0.081 M K_2HPO_4 and 0.019 M KH_2PO_4; pH 7.4). A 25% homogenate (w:v) was prepared by using a Teflon glass Potter–Elvehjem homogenizer (16 complete passes with the pestle), and the microsomal fraction was obtained by differential centrifugation [24]. The microsomal pellet was resuspended in 0.1 M phosphate buffer so that the final concentration was equivalent to 500 mg lung tissue/ml. The suspensions were either used fresh for enzyme assay procedures or quick frozen in liquid nitrogen and stored at −80° and used within a week. No differences in enzyme activities were seen between fresh and frozen microsomes.

For determination of benzo[*a*]pyrene hydroxylase activities, the production of 3- and 9-OH-BP was measured fluorometrically using the method of Nebert and Gelboin [9]. Reaction mixtures (total volume of 1 ml; pH 7.4) contained 0.9 ml of 0.1 M phosphate buffer, bovine serum albumin (BSA) (0.5 mg/ml), NADPH (0.4 mg/ml) (Sigma Chemical Co., St. Louis, MO), $MgCl_2$ (0.61 mg/ml) and 0.1 ml microsomal suspension. BP (Sigma Chemical Co.), dissolved in ethanol (Publicker Manufacturers, Perry Point, MD), acetone, methanol, ethyl acetate or DMSO (all from Fisher Chemical Co., Fair Lawn, NJ) at a final concentration of 2.5 mg/ml, was delivered to the microsomes in a volume of 10 μl. In some cases, solvent volume was increased to a total volume of 50 μl/ml in reactions initiated with BP in 10 μl of solvent vehicle. Metabolites were first extracted in 3.25 ml of hexane, and then 1 ml of the organic phase was extracted in 3 ml NaOH. The amount of 3- and 9-OH-BP in the alkali extractable fraction was determined by measuring fluorescence with a Turner spectrofluorometer (model 430) set at an activation wavelength of 396 nm and an emission wavelength of 522 nm. Quinine sulfate calibrated against 3-OH-BP was employed as the standard.

HPLC assay procedures were similar to those of Selkirk *et al.* [11]. Reaction mixtures (total volume of 5 ml) consisted of 4.5 ml of 0.1 M phosphate buffer containing NADPH (0.4 mg/ml), BSA (0.5 mg/ml), $MgCl_2$ (0.61 mg/ml) and 0.5 ml of microsomal suspension. BP was added to this mixture in 250 μl of DMSO, acetone, methanol, ethanol, or ethyl acetate (final concentration = 0.5 mg/ml) or in 50 μl of each solvent (final concentration = 2.5 mg/ml). Thus, the solvent volume:total reaction volume ratio was proportional to that used in the fluorometric determination of benzo[*a*]pyrene hydroxylase activity. Ethyl acetate extractable metabolites were evaporated under nitrogen gas at room temperature and redissolved in 200 μl of HPLC grade methanol.

HPLC analyses were performed using a Waters System (model 204, Waters Associates, Milford, MA) equipped with a systems controller (model 720), data module (model 730), WISP (automatic sample injection, model 710B), solvent programmer (model 660), fixed wavelength detector (model 440) and a Z-Module Radial Compression Separation System. Separation of metabolites was achieved with an 8MB-C_{18}-10 μ Radial Pak Cartridge and a linear gradient of 65% to 100% methanol over 30 min. Solvent flow rate was 2.0 ml/min, and the eluent was monitored by absorbance at 254 nm with the detector sensitivity setting of 0.05 (AUFS). For identification of BP metabolites, the retention times of sample profiles were compared with those produced by nine authentic standards: BP-9,10-diol, BP-7,8-diol, BP-4,5-diol, 3-OH-BP, 9-OH-BP, BP-1,6-dione, BP-3,6-dione, BP-6,12-dione and BP, which were donated by the IIT Research Institute Chemical Repository, Chicago, IL.

RESULTS

Effects of solvent vehicles on benzo[a]pyrene hydroxylase activity. The effects of the five organic solvent vehicles (DMSO, methanol, ethanol, acetone and ethyl acetate) on benzo[*a*]pyrene hydroxylase activity were evaluated. All reactions were carried out for 20 min in 1 ml of reaction mixture containing lung microsomes equivalent to 50 mg lung tissue. These conditions were consistent with linear production of 3- and 9-OH-BP in the presence of all five solvent vehicles. A constant amount of BP (final concn = 2.5 mg/ml) was delivered to the microsomes in solvent volumes ranging from 10 to 50 μl. This concentration of BP was chosen because it represented the limit of solubility in ethanol and methanol at the lowest solvent vehicle volume.

The effects of varying the solvent vehicle volume on benzo[*a*]pyrene hydroxylase activity are shown in Fig. 1. When BP was delivered in 10 μl of each solvent, the resulting benzo[*a*]pyrene hydroxylase activities as related to solvent type from highest to lowest were: DMSO ≥ methanol > ethanol ≥ acetone > ethyl acetate. The benzo[*a*]pyrene hydroxylase activities in the presence of ethyl acetate are not shown because they were so low that, in most instances, no activity was detected. As the solvent volumes were increased, a dose-dependent inhibition in benzo[*a*]pyrene hydroxylase activity was produced in each case. At the highest solvent volume used, i.e. 50 μl/ml of reaction mixture, the resulting benzo[*a*]pyrene hydroxylase activities were significantly lower than those observed at the 10 μl volume. In this case, the benzo[*a*]pyrene hydroxylase activities as related to the solvents, from highest to lowest, were: DMSO > methanol > acetone ≥ ethanol > ethyl acetate. In general, these findings indicate that benzo[*a*]pyrene hydroxylase activity is inhibited by each solvent in a dose-dependent manner. Also, DMSO appears to be the solvent vehicle which yields the highest activity.

In order to compare the relative inhibition produced by each solvent, the effects of varying the solvent vehicle volumes on benzo[*a*]pyrene hydroxylase activity were determined when all reactions were initiated with BP in 10 μl of DMSO. The results, shown in Fig. 2, indicate a dose-dependent inhibition of benzo[*a*]pyrene hydroxylase produced by each

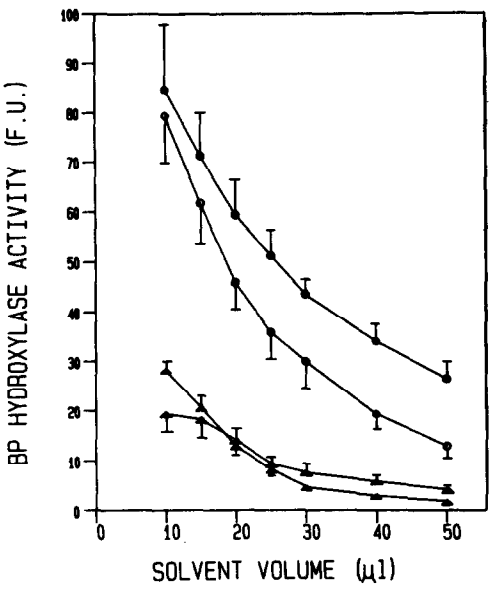

Fig. 1. Effects of varying solvent vehicle volumes on benzo[a]pyrene hydroxylase activity. Benzo[a]pyrene hydroxylase activity was determined by measuring the production of 3- and 9-OH-BP with a fluorometric method. Reaction mixtures (total volume of 1 ml) contained NADPH (0.4 mg/ml), BSA (0.5 mg/ml), $MgCl_2$ (0.61 mg/ml) and 0.1 ml microsomal suspension equivalent to 50 mg of lung tissue in 0.1 M phosphate buffer (pH 7.4). Reaction vessels containing from 0 to 40 μl of each solvent were equilibrated and brought to equal volume by addition of phosphate buffer. Reactions were initiated by adding BP in 10 μl of each solvent (final conc = 2.5 mg/ml) and incubated for 20 min at 37°. Key: (●) DMSO, (○) methanol, (▲) acetone and (△) ethanol. Solvent volumes on the horizontal axis represent final volumes in the reaction vessels. Each point represents the mean for five experiments and the bars are standard errors of the means.

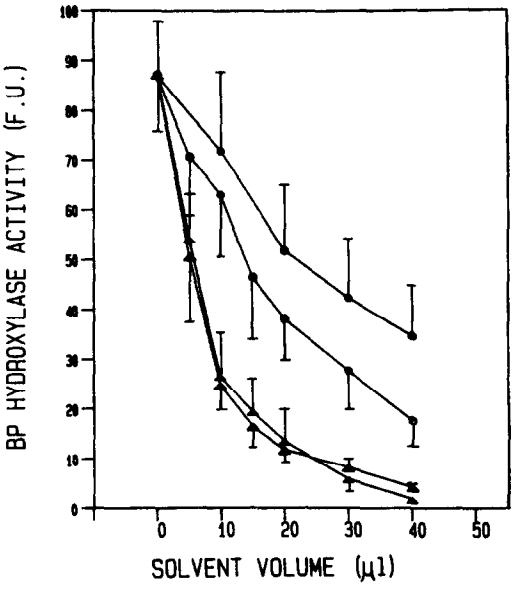

Fig. 2. Effects of solvents on benzo[a]pyrene hydroxylase activity when reactions were initiated with DMSO as the solvent vehicle. Reaction mixtures (total volume of 1 ml) contained NADPH (0.4 mg/ml), BSA (0.5 mg/ml), $MgCl_2$ (0.61 mg/ml) and 0.1 ml microsomal suspension equivalent to 50 mg of lung tissue in 0.1 M phosphate buffer (pH 7.4). Reaction vessels were equilibrated with various volumes of each solvent and brought to equal volume by addition of phosphate buffer. Reactions were then initiated by addition of BP (2.5 mg/ml) in 10 μl DMSO. Zero on the solvent volume axis represents reaction mixtures with 10 μl of DMSO only. Key: (●) DMSO, (○) methanol, (▲) acetone and (△) ethanol. Other values on the same axis represent the solvent volume in addition to 10 μl of DMSO. Each point represents the mean for five experiments and the bars are standard errors of the means.

solvent. The $K_{1/2}$ values, i.e. the solvent volumes producing 50% inhibition, were obtained from double-reciprocal plots and are listed in Table 1. These values indicate that the degree of inhibition produced by the solvents, from the least effective to the most effective inhibitor, was: DMSO > methanol > acetone ≥ ethanol.

It is possible that DMSO is stimulatory to benzo[a]pyrene hydroxylase. To test this possibility, DMSO was added in increasing volumes to reaction mixtures in which the BP was delivered in ethanol, i.e. the solvent vehicle which was most inhibitory to enzyme activity. These data are not presented. There was a decline in benzo[a]pyrene hydroxylase activity with increasing amounts of DMSO, indicating that the solvent vehicle did not stimulate enzyme activity under these conditions. Therefore, these data taken together indicate that all solvents were inhibitory to benzo[a]pyrene hydroxylase and that activities were least affected when BP was delivered in DMSO.

Effects of solvent vehicles on the benzo[a]pyrene metabolite profile. The results presented thus far show that production of 3- and 9-OH-BP was affected by the type and volume of solvent vehicle used. Therefore, HPLC analyses were performed to determine if the solvents affected production of other BP metabolites. Since benzo[a]pyrene hydroxylase activities were the highest with DMSO as the solvent vehicle, the separation conditions were determined using this solvent. For these experiments, 20-min incubation times and microsomal suspensions equivalent to 250 mg lung tissue were used. These con-

Table 1. Volumes of solvent vehicles which produce 50% inhibition of benzo[a]pyrene hydroxylase activity ($K_{1/2}$)

Solvent	$K_{1/2}$ (μl)
DMSO	25.30 ± 3.14
Methanol	15.30 ± 2.07
Acetone	7.90 ± 0.78
Ethanol	6.70 ± 0.54

Data from Fig. 2 were plotted using double-reciprocal plots. The data were fitted with straight lines by linear regression analysis to obtain $K_{1/2}$ values, i.e. the solvent volumes required to produce a 50% reduction in benzo [a]pyrene hydroxylase activity. Each number represents the mean value ± S.E.M. for five determinations.

Table 2. Effects of solvent vehicles on production of BP metabolites

Solvent vehicle	Metabolite production (pmoles/min/g lung tissue)				
	9,10-Diol	4,5-Diol	7,8-Diol	Quinones	3-OH-BP
DMSO	21.4 ± 3.8	42.0 ± 6.9	21.8 ± 1.4	16.6 ± 4.5	555.4 ± 83.5
Methanol	14.9 ± 2.4	20.4 ± 1.6	10.2 ± 0.3	12.7 ± 1.2	230.0 ± 24.6
Acetone	13.9 ± 2.2	24.4 ± 1.9	11.1 ± 1.6	22.4 ± 1.1	75.3 ± 11.5
Ethyl acetate	4.3 ± 0.5	ND*	ND	ND	7.1 ± 3.9
Ethanol	13.2 ± 1.2	ND	ND	ND	42.7 ± 4.3

Reaction mixtures consisted of 4.5 ml of 0.1 M phosphate buffer (pH 7.4) containing $MgCl_2$ (0.61 mg/ml), NADPH (0.4 mg/ml) and BSA (0.5 mg/ml). Microsomal suspension (0.5 ml) equivalent to 250 mg lung tissue was added, and reactions were initiated with BP in 250 μl of each solvent at a concentration of 1 mg/ml. The values for DMSO, methanol and acetone were calculated from 20-min reaction times. The values for ethanol and ethyl acetate were calculated from 60-min reaction times. Each number represents the mean value ± S.E.M. for six experiments.

*ND = metabolites not detected.

ditions were consistent with linear formation of metabolites.

When DMSO was employed as the solvent vehicle, the major metabolite in the profile was a phenol peak which eluted at a time similar to the 3-OH-BP standard (R_t = 15.1 min). This peak constituted 80% of total metabolite production. Although this single phenol peak eluted with a retention time similar to the 3-OH-BP standard, it is likely that other BP phenols contributed to its formation but could not be separated under these conditions [22, 25]. In all chromatograms, 9-OH-BP was not detected as a metabolite. Approximately 16% of the total metabolite profile could be accounted for by three BP diols, BP-4,5-diol (R_t = 7.5 min), BP-7,8-diol (R_t = 8.4 min) and BP-9,10-diol (R_t = 4.3 min). BP-4,5-diol was the major diol component. The fact that the formation of these peaks was reduced significantly (by approximately 80%) in the presence of 0.25 mM trichloropropene oxide (TCPO), an epoxide hydrase inhibitor, was further evidence that they were BP diols. Two peaks, which represented the remaining 4% of the total metabolites, were identified as BP-1,6-quinone (R_t = 11.6 min) and BP-3,6-quinone (R_t = 12.4 min). In certain animals, where overall metabolite production was higher than in others, BP-6,12-quinone (R_t = 10.3 min) was also detected in the metabolite profile.

The effects of the other four solvent vehicles, methanol, ethanol, acetone and ethyl acetate, on the BP metabolite profile were studied. The data from these studies are shown in Table 2. The three BP diols, 3-OH-BP, and BP quinones were all produced in the presence of DMSO, methanol and acetone. In general, production of all metabolites was the greatest with DMSO except for quinone formation which was the greatest when acetone was used as the solvent vehicle. When BP was delivered in either ethanol or ethyl acetate, metabolite production was restricted to 3-OH-BP and BP-9,10-diol. These results show that the type of solvent used for solubilizing BP quantitatively affects the BP metabolite profile.

To determine if the solvent vehicles affected the distribution of metabolites, the data were expressed as a percentage of the total metabolite production. These results are shown in Table 3. When DMSO or methanol was used, the BP metabolite ratios were similar. The metabolite profile was altered, however, when acetone was used as the solvent. In this case, there was a significant reduction in 3-OH-BP production concomitant with a significant increase in BP-4,5-diol and total quinone formation. BP-9,10-diol and 3-OH-BP were the only metabolites produced in the presence of ethanol and ethyl acetate, and they were produced in similar proportions.

Table 3. Effects of solvent vehicles on the distribution of BP metabolites

Solvent vehicle	BP metabolites (% of total)				
	9,10-Diol	4,5-Diol	7,8-Diol	Quinones	3-OH-BP
DMSO	4.4 ± 0.9	7.7 ± 0.9	4.9 ± 0.6	3.5 ± 0.5	79.6 ± 2.0
Methanol	6.4 ± 0.9	8.4 ± 0.8	5.0 ± 0.5	5.6 ± 0.5	74.6 ± 1.3
Acetone	6.6 ± 0.7	11.2 ± 0.4*	6.0 ± 0.8	11.0 ± 0.6*	65.2 ± 0.8*
Ethyl acetate	22.0 ± 2.7	ND†	ND	ND	78.0 ± 2.7
Ethanol	23.7 ± 1.8	ND	ND	ND	76.3 ± 1.8

These data were recalculated from those presented in Table 2. The areas under the peaks on the chromatograms for the five principal types of BP metabolites are expressed as a percentage of the total. The values for DMSO, methanol and acetone were calculated from 20-min reaction times and those for ethyl acetate and ethanol were calculated from 60-min reaction times. Each value represents the mean ± S.E.M. for six experiments.

* Significantly different from corresponding DMSO and methanol ($P < 0.05$).

† ND = metabolites not detected.

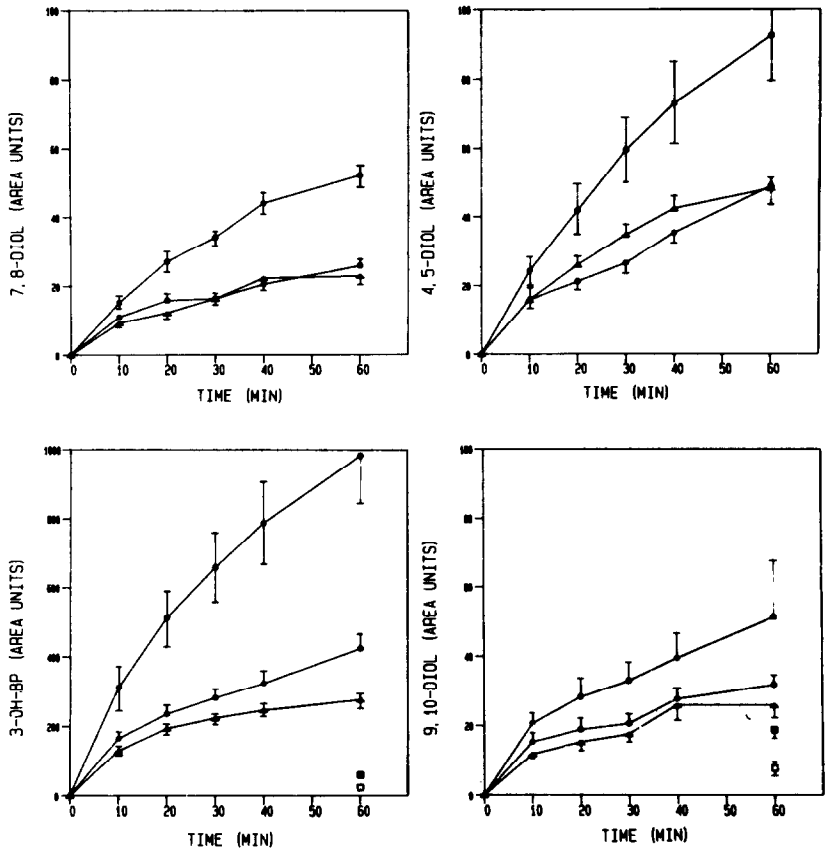

Fig. 3. Time courses for production of BP metabolites in the presence of three different solvent vehicles: DMSO (●), methanol (○) and acetone (▲). Metabolite production is expressed in terms of areas under the peaks from chromatograms. Metabolism in the presence of ethanol (■) and ethyl acetate (□) are shown only at 60-min reaction times for the BP-9,10-diol and 3-OH-BP. The area increments for 3-OH-BP are also 10× larger than those in the other figures. Points on the graphs represent the means of areas from chromatograms of six experiments and the bars are standard errors of the means.

These results indicate that the choice of solvent qualitatively alters the BP metabolite profile produced by rabbit lung microsomes.

Time courses for production of some BP metabolites in the presence of various solvent vehicles are shown in Fig. 3. At each time point, production of all metabolites was highest when DMSO was used as the solvent vehicle. Also, linearity of product formation for 3-OH-BP, BP-7,8-diol, and BP-4,5-diol was retained through 20-min incubation times with DMSO. On the other hand, linearity was lost after 10 min with acetone or methanol as the solvent vehicle. Since quinone formation was not clearly resolved at shorter incubation times, it is not shown in Fig. 3. These results indicate that differences in the magnitudes and time courses of metabolite production occurred when different solvent vehicles were used.

DISCUSSION

The data presented here indicate that BP metabolism in rabbit lung microsomes was affected by the organic solvent vehicle which is used to solubilize BP for the enzyme assay. All solvents tested were inhibitory to benzo[a]pyrene hydroxylase in a dose-dependent manner. The choice of solvent vehicle affected the time course and magnitude of formation of all BP metabolites. Solvent-dependent variations were also seen in the types of BP metabolites produced. The results indicate that DMSO is the solvent vehicle of choice for studying BP metabolism in rabbit lung microsome since the magnitude of production for all metabolites was greatest and the linearity of product formation was retained the longest in the presence of this solvent vehicle.

Although we have found that DMSO is the solvent of choice in lung, other investigators routinely use acetone as a solvent vehicle for the evaluation of pulmonary benzo[a]pyrene hydroxylase activity or BP metabolism [13–17]. To determine if the higher activity produced by DMSO was a phenomenon restricted to lung, benzo[a]pyrene hydroxylase activity was evaluated in rabbit liver employing DMSO or acetone as BP solvents. In the liver, higher benzo[a]pyrene hydroxylase activities resulted when acetone was used (data not shown). Thus, the solvent effects appear to be organ-specific. These findings also suggest that benzo[a]pyrene hydroxylase activities in lung relative to liver may have been under-

estimated by other investigators. In the present study, benzo[a]pyrene hydroxylase activities evaluated with acetone as the solvent vehicle indicate that the liver is eleven times more active than the lung. However, if the liver values are compared with lung values when DMSO was used as the solvent vehicle, the liver is only three times more active than the lung.

Our data also demonstrate that the volume of solvent vehicle significantly influenced the metabolism of BP. Most investigators use 40 or 50 μl of solvent vehicle per ml of reaction mixture to study pulmonary BP hydroxylase activity [14, 19, 20, 26]. However, we found that, when BP was delivered in 10 μl of each solvent, the enzyme activities were significantly higher than when a 50 μl volume was used (see Fig. 1). Similar results were obtained with production of all BP metabolites, i.e. the amounts formed were greater at solvent vehicle volumes of 10 μl than at 50 μl (data not shown). The effect of solvent volume on BP metabolite production was the most pronounced when ethanol was used as the vehicle. At the 50 μl volume, metabolite production was restricted to the 9,10-diol and 3-OH-BP. However, at the 10 μl volume, all metabolites were present and the profile was similar to those produced in the presence of DMSO or methanol (data not presented). Thus, the solvent vehicle volume can influence enzyme activity and BP metabolite production in the lung.

The type of organic solvent vehicle used also influenced the distribution of metabolites. In the presence of DMSO or methanol, the metabolites produced, as a percentage of the total, were 3-OH-BP (77%), diols (17%), and quinones (6%). However, when acetone was used as the solvent vehicle, the metabolite profile was altered, i.e. there was a reduction in 3-OH-BP along with enhanced production of the 4,5-diol and quinones. The metabolite pattern we obtained with acetone is somewhat similar to BP metabolite profiles obtained by other investigators in rabbit lung microsomes [27] and one purified form of P-450 isolated from rabbit lung microsomes [17] where acetone was also employed as the solvent vehicle. In both of these other studies, however, greater amounts of BP-9,10-diol, BP-4,5-diol and BP quinones were reported. These findings indicate that the type of solvent selectively affects metabolite production and thus the type of BP metabolite profile produced by the rabbit lung may be solvent dependent.

Were the effects of the organic solvent vehicles related to the solubilities of BP in these solvents? The order of BP solubilities, from greatest to least, is ethyl acetate > acetone > DMSO > ethanol > methanol. However, there was no relationship between the order of solubilities and the effects of the solvents on BP hydroxylase activity. Therefore, the solvent effects on BP metabolism are not related to solubility.

Since delivery of solvent vehicles in equal volumes may lead to different solvent molarities in the final reaction mixture, the relationship between solvent vehicle molarity and enzyme activity was evaluated. Enzyme activities were compared at points of equal molarity on the solvent inhibition curve (Fig. 1). The resulting benzo[a]pyrene hydroxylase activities as related to each solvent from highest to lowest were methanol > DMSO > acetone ≥ ethanol. Thus, when enzyme activity is evaluated with respect to solvent molarity, methanol appears to be less inhibitory than DMSO, but the relative activities obtained with ethanol or acetone are unaffected.

Although the mechanisms for solvent inhibition have not been investigated in the present study, competitive inhibition, or the solvent competing for the active site on the enzyme, is one possible mechanism which has been suggested in other studies [7, 28]. It is also likely that solvents may affect allosteric sites on the P-450 molecule, thus producing conformational changes in enzyme protein. Since multiple forms of P-450 have been identified in rabbit lung microsomes [17, 29], it is possible that solvents may selectively affect each P-450 form. The MFO-related enzymes, epoxide hydrase and NADPH cytochrome P-450 reductase, may also be sensitive to organic solvents. Thus, the differences in the magnitude of metabolite production and the alterations in the BP metabolite profile produced by various solvents in the present study may be the result of one or a combination of the above factors.

In conclusion, we have shown that BP metabolism in rabbit lung microsomes is affected by the type of organic solvent used to deliver BP to the microsomes. Since all solvents produced a volume-dependent inhibition of benzo[a]pyrene hydroxylase, smaller volumes appear more feasible to use since they are less inhibitory to the enzyme. These factors may be important in assessing basal benzo[a]pyrene hydroxylase activity in the lung or in other tissues where low benzo[a]pyrene hydroxylase activities have been reported. In the evaluation of BP metabolite production, solvents were shown to affect the magnitude and time course of metabolite formation and produce variations in the metabolite profile. These factors are important since the production of potentially carcinogenic BP metabolites may be underestimated. Thus, it is important that the choice of solvent and delivery volume be considered when BP metabolism is evaluated in any tissue.

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