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SPECIES DIFFERENCES IN THE EFFECT OF MORPHINE ADMINISTRATION OR ADRENALECTOMY ON THE SUBSTRATE INTERACTIONS WITH CYTOCHROME P-450 AND DRUG OXIDATIONS BY LIVER MICROSOMES

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Abstract—The hexobarbital hydroxylation and the magnitude of hexobarbital interaction with cytochrome P-450 were markedly decreased in liver microsomes from morphine-treated male rats, whereas they were not decreased in liver microsomes from morphine-treated female rats, male and female mice and rabbits. The binding capacity of cytochrome P-450 for hexobarbital calculated from the magnitude of hexobarbital interaction with cytochrome P-450 in liver microsomes was also decreased only in the morphine-treated male rats.

Similarly, the hexobarbital hydroxylation, the magnitude of hexobarbital interaction with cytochrome P-450 and the binding capacity of cytochrome P-450 in liver microsomes were decreased only in adrenalectomized male rats, but not in adrenalectomized female rats, male and female mice.

In contrast to the results obtained with hexobarbital, the aniline hydroxylation, the magnitude of aniline interaction with cytochrome P-450 and the binding capacity of cytochrome P-450 for aniline were not decreased in liver microsomes from male and female rats, mice and rabbits by the morphine treatment or adrenalectomy.

These results therefore support the previous suggestion that the decrease in the hexobarbital hydroxylation and in the magnitude of hexobarbital interaction with cytochrome P-450 is mainly due to the decrease in the binding capacity of cytochrome P-450 for hexobarbital, probably through an impairment of the action of androgen to increase the binding capacity of cytochrome P-450 for hexobarbital.

IT WAS observed that the activity of drug-metabolizing enzymes in liver microsomes was decreased in morphine-treated or adrenalectomized rats.[1–3] In a previous paper,[3] we reported that the hexobarbital hydroxylation and aminopyrine *N*-demethylation in liver microsomes were decreased by the morphine treatment or adrenalectomy in male rats, but not in female rats. On the other hand, the aniline and zoxazolamine hydroxylations in liver microsomes were not decreased by the morphine treatment or adrenalectomy in both male and female rats.[3] The different effect of the morphine treatment or adrenalectomy appeared to be related to the fact that the hexobarbital hydroxylation and aminopyrine *N*-demethylation are markedly stimulated by androgen, whereas the aniline and zoxazolamine hydroxylations are not.[3,4] From these results and others, it has been supposed that the decrease in the hexobarbital hydroxylation and aminopyrine *N*-demethylation in liver microsomes from morphine-treated or adrenalectomized rats is due to an impairment of the mechanism of androgen stimulation for these oxidative activities.[3,4]

On the other hand, the hexobarbital hydroxylation and aminopyrine *N*-demethylation in liver microsomes of mice and rabbits were not stimulated by androgen, and the lack of the mechanism of androgen-dependent stimulation for the drug-metabolizing enzymes has been suggested.[5,6]

Several lines of evidence have established that a unique hemoprotein called P-450 is involved as the oxygen activating component and the substrate-binding site in a number of monooxygenase reactions such as the hydroxylations of drugs and steroid hormones by liver microsomes[7,8] and the magnitude of the substrate binding with cytochrome P-450 is one of the important factors controlling the rate of the over-all hydroxylation by liver microsomes.[6,9,10] The magnitude of the spectral change induced by hexobarbital was greater in liver microsomes from male rats than in those from female rats and androgen administration increased the magnitude of the spectral change in castrated male and female rats.[6,9] On the other hand, there was no clear sex difference in the magnitude of hexobarbital-induced spectral change in liver microsomes from mice and rabbits and the magnitude of the spectral change was not stimulated by the administration of androgen.[5,6]

In previous papers,[11,12] it has been demonstrated that morphine administration or adrenalectomy markedly decreased the magnitude of spectral change induced by hexobarbital in liver microsomes from male rats, but not in those from female rats. It is therefore reasonable to suppose that, if the decrease in the hexobarbital hydroxylation and in the magnitude of hexobarbital-induced spectral change by morphine administration or adrenalectomy is due to an impairment of androgen action which increases the hexobarbital hydroxylation and magnitude of the spectral change, the morphine administration or adrenalectomy may not produce significant decrease in the hexobarbital hydroxylation and magnitude of the spectral change in male mice and rabbits. In the present studies, this possibility was investigated with both sexes of rats, mice and rabbits.

MATERIALS AND METHODS

Male and female rats of the Wistar strain, weighing about 190 and 160 g, respectively, male and female mice of the dd strain, about 25 and 22 g, respectively, and male and female rabbits, about 2·3 and 2·0 kg, respectively, were used.

The rats, mice and rabbits were treated subcutaneously with three increasing doses of morphine 72, 48 and 24 hr before sacrifice. The doses of morphine were as follows: rats, 30, 35 and 40 mg/kg; mice, 110, 120 and 130 mg/kg; rabbits, 60, 70 and 80 mg/kg. The rats and mice were adrenalectomized 5 days before experiments and given 1% sodium chloride solution as drinking water.

The hepatic 10,000 *g* supernatant and microsomal fractions and incubation mixture were prepared as described in the previous paper.[5] The hexobarbital hydroxylation was determined by the disappearance of the substrate according to the method of Cooper and Brodie,[13] the aminopyrine *N*-demethylation was determined by measuring 4-aminoantipyrine formed according to the method of La Du *et al.*,[14] and the aniline hydroxylation was determined by the formation of *p*-aminophenol according to the method described by Kato and Gillette.[4]

Microsomal protein content was measured by the method of Lowry *et al.*[15] The cytochrome P-450 content was determined by the difference spectrum induced by carbon monoxide as described in previous papers.[10,16]

The spectral change induced by hexobarbital or aniline was determined as described in previous papers.[6,10] The concentration of hexobarbital or aniline was 1·6 and 2·0 mM and microsomal protein concentration was about 1·2 mg/ml.

RESULTS

Effect of morphine administration or adrenalectomy on the microsomal protein and cytochrome P-450 contents

The administration of morphine or adrenalectomy did not decrease the content of hepatic microsomal protein in male and female rats, mice and rabbits. In addition, no clear sex difference and species difference were observed concerning the microsomal protein content.

The cytochrome P-450 content in liver microsomes from male and female rats was 0·91 ± 0·04 and 0·70 ± 0·04 mμmole/mg microsomal protein, respectively, whereas that of male mice, female mice, male rabbits and female rabbits was 0·91 ± 0·05, 0·94 ± 0·05, 1·74 ± 0·07 and 1·69 ± 0·10 mμmole/mg microsomal protein, respectively. The administration of morphine or adrenalectomy significantly (18 or 17 per cent, respectively) decreased the cytochrome P-450 content in male rats but not in female rats and male and female mice and rabbits.

Effect of morphine administration on the hexobarbital or aniline hydroxylation in liver microsomes

As reported in the previous paper,[5] clear sex difference in the hexobarbital hydroxylation was observed only in rats, but not in mice and rabbits (Table 1). The hexobarbital hydroxylation in liver microsomes from the morphine-treated animals was

TABLE 1. EFFECT OF MORPHINE ADMINISTRATION ON THE HEXOBARBITAL HYDROXYLATION BY LIVER MICROSOMES

Species	Sex	Hexobarbital hydroxylation		Difference (%)
		Control	Morphine	
		(mμmole/mg protein/30 min)		
Rats	M	97·2 ± 6·0 (8)	48·6 ± 4·2 (8)	−50*
	F	36·3 ± 2·2 (8)	33·0 ± 2·9 (7)	− 9
Mice	M	39·1 ± 3·4 (6)	38·1 ± 3·0 (6)	− 2
	F	40·9 ± 2·9 (6)	42·5 ± 3·5 (6)	+ 4
Rabbits	M	78·0 ± 6·8 (8)	77·2 ± 5·2 (8)	− 1
	F	79·6 ± 4·5 (8)	80·4 ± 4·8 (8)	+ 1
		(mμmole/mμmole P-450/30 min)		
Rats	M	105·7 ± 5·9 (8)	64·5 ± 5·0 (8)	−39*
	F	51·9 ± 2·8 (8)	49·3 ± 3·8 (7)	− 5
Mice	M	42·5 ± 3·1 (6)	40·4 ± 2·6 (6)	− 5
	F	41·3 ± 4·0 (6)	44·1 ± 3·7 (6)	+ 7
Rabbits	M	44·8 ± 3·1 (8)	46·5 ± 4·1 (8)	+ 4
	F	47·1 ± 2·9 (8)	45·9 ± 2·9 (8)	− 3

Morphine was given subcutaneously 72, 48 and 24 hr before sacrifice. The doses of morphine were as follows: rats (30, 35 and 40 mg/kg); mice (110, 120 and 130 mg/kg); rabbits (60, 70 and 80 mg/kg). The results are expressed as mean ± S.E. The figures in parentheses indicate number of the determination. Pooled livers from three mice were used for each determination. The asterisks indicate the significant difference ($P < 0·05$) from control values.

clearly decreased in male rats, but not in female rats and both sexes of mice and rabbits (Table 1). Similarly, the hexobarbital hydroxylation per unit of cytochrome P-450 was decreased only in microsomes from morphine-treated male rats, but not in those from the other morphine-treated animals. In addition, similar results were obtained with aminopyrine *N*-demethylation.

In contrast to the results obtained with the hexobarbital hydroxylation, the morphine treatment did not significantly decrease the aniline hydroxylation in male rats as well as in female rats and both sexes of mice and rabbits (Table 2).

TABLE 2. EFFECT OF MORPHINE ADMINISTRATION ON THE ANILINE HYDROXYLATION BY LIVER MICROSOMES

Species	Sex	Aniline hydroxylation		Difference (%)
		Control	Morphine	
		(mμmole/mg protein/30 min)		
Rats	M	20·4 ± 1·3 (8)	17·6 ± 1·3 (8)	−13
	F	15·6 ± 1·0 (8)	14·4 ± 1·3 (8)	− 8
Mice	M	34·5 ± 1·5 (6)	34·2 ± 2·5 (6)	− 1
	F	34·9 ± 2·1 (6)	36·6 ± 2·3 (6)	+ 5
Rabbits	M	23·7 ± 2·0 (8)	24·2 ± 2·0 (8)	+ 2
	F	23·4 ± 1·4 (8)	22·9 ± 1·7 (8)	− 2
		(mμmole/mμmole P-450/30 min)		
Rats	M	22·2 ± 1·7 (8)	23·4 ± 1·5 (8)	+ 5
	F	22·3 ± 1·3 (8)	21·5 ± 1·2 (8)	− 4
Mice	M	37·5 ± 1·8 (6)	36·0 ± 2·1 (6)	− 4
	F	35·6 ± 1·9 (6)	38·5 ± 2·0 (6)	+ 8
Rabbits	M	13·6 ± 1·0 (8)	14·6 ± 1·3 (8)	+ 7
	F	13·8 ± 0·6 (8)	13·1 ± 1·1 (8)	− 5

See the legends for Table 1.

Effect of adrenalectomy on the hexobarbital or aniline hydroxylation in liver microsomes

The hexobarbital hydroxylation was decreased in liver microsomes from the adrenalectomized male rats, but not in those from adrenalectomized female rats and male and female mice (Table 3). Similarly, the hexobarbital hydroxylation per unit of cytochrome P-450 was decreased only in microsomes from the adrenalectomized male rats, but not in those from the other adrenalectomized animals.

However, the adrenalectomy did not significantly decrease the aniline hydroxylation by liver microsomes in the male rats as well as in the female rats and male and female mice.

Effect of morphine administration on the magnitude of spectral change induced by hexobarbital or aniline in liver microsomes

As shown in Table 4, clear sex difference in the magnitude of spectral change induced by hexobarbital per unit of microsomal protein was observed only in rats, but not in mice and rabbits. The morphine treatment decreased the magnitude of the spectral change in liver microsomes from male rats, but not in those from female rats

TABLE 3. EFFECT OF ADRENALECTOMY ON THE HEXOBARBITAL HYDROXYLATION BY LIVER MICROSOMES

Species	Sex	Hexobarbital hydroxylation: Control	Hexobarbital hydroxylation: Adrenalectomy	Difference (%)
		(mμmole/mg protein/30 min)		
Rats	M	100·2 ± 7·0 (10)	49·1 ± 4·3 (10)	−51*
	F	38·7 ± 3·4 (10)	33·6 ± 3·0 (10)	−13
Mice	M	42·0 ± 4·0 (7)	44·1 ± 3·8 (7)	+ 5
	F	43·6 ± 3·5 (7)	40·5 ± 4·1 (7)	− 7
		(mμmole/mμmole P-450/30 min)		
Rats	M	111·3 ± 8·3 (10)	65·3 ± 5·5 (10)	−40*
	F	56·0 ± 4·6 (10)	54·1 ± 4·1 (10)	− 3
Mice	M	46·9 ± 3·5 (7)	51·2 ± 3·9 (7)	+10
	F	47·4 ± 4·0 (7)	47·6 ± 4·3 (7)	+ 1

The rats and mice were adrenalectomized 5 days before sacrifice. The results are expressed as means ± S.E. The figures in parentheses indicate number of the determination. Pooled livers from three mice were used for each determination. The asterisks indicate the significant difference ($P < 0.05$) from control values.

and both sexes of mice and rabbits. Similarly, the magnitude of spectral change induced by hexobarbital per unit of cytochrome P-450 was decreased only in liver microsomes from morphine-treated male rats, but not in those from the other animals.

TABLE 4. EFFECT OF MORPHINE ADMINISTRATION ON THE MAGNITUDE OF SPECTRAL CHANGE INDUCED BY HEXOBARBITAL IN LIVER MICROSOMES

Species	Sex	Hexobarbital-induced spectral change: Control	Hexobarbital-induced spectral change: Morphine	Difference (%)
		($\Delta A \times 10^3$/mg protein/ml)		
Rats	M	18·5 ± 1·2 (8)	8·8 ± 0·9 (8)	−53*
	F	6·7 ± 0·5 (8)	5·8 ± 0·4 (7)	−14
Mice	M	7·8 ± 0·6 (6)	7·0 ± 0·6 (6)	−10
	F	8·2 ± 0·5 (6)	7·9 ± 0·7 (6)	− 4
Rabbits	M	15·7 ± 1·0 (8)	15·5 ± 1·3 (8)	− 1
	F	15·3 ± 1·1 (8)	15·0 ± 1·2 (8)	− 2
		($\Delta A \times 10^3$/mμmole P-450/ml)		
Rats	M	20·1 ± 1·1 (8)	11·7 ± 0·9 (8)	−42*
	F	9·5 ± 0·5 (8)	8·6 ± 0·5 (8)	− 9
Mice	M	8·6 ± 0·7 (6)	7·4 ± 0·3 (6)	−13
	F	8·5 ± 0·4 (6)	8·3 ± 0·5 (6)	− 2
Rabbits	M	9·0 ± 0·5 (8)	9·3 ± 0·7 (8)	+ 3
	F	9·1 ± 0·4 (8)	8·6 ± 0·5 (8)	− 5

The magnitude of the spectral change is determined by the decrement of the absorbance between 421 mμ and 500 mμ produced on addition of hexobarbital into a microsomal suspension and expressed as the change of absorbance per mg protein/ml or per mμmole P-450/ml.

See the legends for Table 1.

These results suggested that the binding capacity of cytochrome P-450 for hexobarbital in liver microsomes was decreased only in male rats by the morphine treatment.

On the other hand, the magnitude of aniline-induced spectral change per unit of microsomal protein and of cytochrome P-450 was not decreased in the morphine-treated male rats as well as in the other animals.

Effect of adrenalectomy on the magnitude of the spectral change induced by hexobarbital or aniline in liver microsomes

The magnitude of spectral change induced by hexobarbital per unit of microsomal protein was decreased only in the adrenalectomized male rats, but not in the adrenalectomized female rats and male and female mice (Table 5). Similarly, the magnitude of the spectral change per unit of cytochrome P-450 was decreased only in liver microsomes from the adrenalectomized male rats.

TABLE 5. EFFECT OF ADRENALECTOMY ON THE MAGNITUDE OF SPECTRAL CHANGE INDUCED BY HEXOBARBITAL IN LIVER MICROSOMES

Species	Sex	Hexobarbital-induced spectral change		
		Control	Adrenalectomy	Difference (%)
		($\Delta A \times 10^3$/mg protein/ml)		
Rats	M	18·9 ± 1·2 (10)	8·7 ± 0·9 (10)	−54*
	F	6·6 ± 0·5 (10)	5·5 ± 0·5 (10)	−16
Mice	M	8·0 ± 0·3 (7)	8·1 ± 0·6 (7)	+ 1
	F	8·4 ± 0·5 (7)	7·9 ± 0·7 (7)	− 6
		($\Delta A \times 10^3$/mμmole P-450/ml)		
Rats	M	21·0 ± 1·2 (10)	11·6 ± 0·8 (10)	−45*
	F	9·3 ± 0·6 (10)	8·9 ± 0·5 (10)	− 4
Mice	M	8·8 ± 0·3 (7)	9·4 ± 0·6 (7)	+ 7
	F	9·1 ± 0·4 (7)	9·3 ± 0·5 (7)	+ 2

See the legends for Table 3 and 4.

However, the adrenalectomy did not significantly decrease the magnitude of aniline-induced spectral change per unit of microsomal protein and of cytochrome P-450 in male rats as well as in the female rats and male and female mice.

DISCUSSION

In previous papers, it has been demonstrated that the magnitude of the spectral change induced by hexobarbital and the hexobarbital hydroxylation by liver microsomes were markedly decreased in morphine-treated or adrenalectomized male rats, but not in morphine-treated or adrenalectomized female rats. [11,12]

On the other hand, in the present studies, the magnitude of hexobarbital-induced spectral change and the hexobarbital hydroxylation by liver microsomes were not decreased in the morphine-treated or adrenalectomized male and female mice or rabbits. Moreover, the magnitude of aniline-induced spectral change and the aniline hydroxylation by liver microsomes were not decreased in the morphine-treated or

adrenalectomized male and female rats, as well as mice and rabbits. These results indicated that the hexobarbital and aniline hydroxylations in liver microsomes are correlated to the magnitude of the interaction of cytochrome P-450 with the drugs.

In addition, it was demonstrated that the binding capacity of cytochrome P-450 for hexobarbital was decreased in liver microsomes from the morphine-treated or adrenalectomized male rats, but not decreased in those from the other animals. The binding capacity of cytochrome P-450 for aniline was not decreased in liver microsomes from the morphine-treated or adrenalectomized rats, mice and rabbits of both sexes.

On the other hand, it has been reported that there is clear sex difference in the hexobarbital hydroxylation, magnitude of hexobarbital interaction with cytochrome P-450 and binding capacity of cytochrome P-450 in liver microsomes from rats, but not in liver microsomes from mice and rabbits.[5,6,9] The administration of androgen to castrated rats increased the hexobarbital hydroxylation, magnitude of hexobarbital interaction with cytochrome P-450 and binding capacity of cytochrome P-450.[6,9]

However, in contrast to the results observed in rats, there is no sex difference in mice and rabbits.[5] Moreover, there is no clear sex difference in the aniline hydroxylation, magnitude of aniline interaction with cytochrome P-450 and binding capacity of cytochrome P-450 for aniline in liver microsomes from rats, mice and rabbits, excepting the existence of a slight sex difference in the aniline hydroxylation and magnitude of aniline interaction with cytochrome P-450 in rat liver microsomes.[5,6,9]

The results obtained in the present studies, therefore, indicate that the observed species difference between male rats and male mice and rabbits concerning the effect of morphine administration or adrenalectomy may be related to the presence of the sex difference in the hexobarbital hydroxylation, magnitude of hexobarbital interaction with cytochrome P-450 and binding capacity of cytochrome P-450 in the intact animals. Moreover, these results support the previous suggestion that the decrease in the hexobarbital hydroxylation and the magnitude of hexobarbital interaction with cytochrome P-450 is mainly due to the decrease in the binding capacity of cytochrome P-450 for hexobarbital probably through an impairment of the action of androgen to increase the binding capacity of cytochrome P-450 for hexobarbital.

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