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DEBRISOQUINE 4-MONOOXYGENASE AND BUFURALOL 1′-MONOOXYGENASE ACTIVITIES IN BOVINE AND RABBIT TISSUES

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Abstract—The tissue distributions of debrisoquine 4-monooxygenase and bufuralol 1′-monooxygenase activities in microsomes from bovine and rabbit tissues were analysed. Debrisoquine 4-monooxygenase and bufuralol 1′-monooxygenase activities were found in liver, and at low levels in cerebral cortex, kidney cortex, lung, small intestine and spleen. Other tissues, such as kidney medulla, adrenocortex, adrenomedulla, blood vessels, thyroid gland, heart, ovary, uterus and testis, showed low levels of bufuralol 1′-monooxygenase activity but no detectable debrisoquine 4-monooxygenase activity. The bufuralol/debrisoquine monooxygenase activity ratios were higher in kidney and lung, and lower in cerebral cortex and spleen than in liver. Both monooxygenase activities in several bovine tissues including liver were inhibited strongly by phenylisocyanide (0.1 mM) and quinidine (0.5 mM), moderately by metyrapone (1 mM), and not at all by KCN (1 mM). NaN_3 (5 mM) and sodium cholate (0.5% w/v) inhibited debrisoquine 4-monooxygenase activity strongly and moderately, but bufuralol 1′-monooxygenase activity moderately and strongly, respectively. No effect of a hydroxyl radical scavenger or of superoxide dismutase on either monooxygenase activity was observed. It was concluded from these results, as well as the NADPH dependency of the reactions, that the two monooxygenase reactions observed in these tissues were catalysed by cytochrome P450s.

Over 25 drugs and chemicals, including debrisoquine, bufuralol, sparteine and dextromethorphan, are metabolized by cytochrome P450 IID [1–4]. Some members of the cytochrome P450 (P450‡) subfamily differing only slightly in amino acid sequence show different substrate specificities [5–8], and are expressed differently in various tissues and at various ages [9, 10]. In rat liver, two members of the P450 IID subfamily, the db1 and db2 proteins, were identified [9]. The former exhibits both debrisoquine 4-monooxygenase and bufuralol 1′-monooxygenase activities (db1 type), and the latter bufuralol 1′-monooxygenase activity but not debrisoquine 4-monooxygenase activity (db2 type) [9]. Similarly, in human liver, the db1 type (P450 bufI) and db2 type (P450 bufII) were identified [3, 10]. Therefore, the debrisoquine 4-monooxygenase reaction is catalysed by P450 db1, while the bufuralol 1′-monooxygenase reaction is catalysed by more than two forms of P450. Usually, several P450s are responsible for the metabolism of a certain drug or chemical [11]. However, the substrate specificity of debrisoquine is marked as it is apparently only metabolized by P450db1 and individuals with defects in the gene for P450db1 were poor metabolizers of debrisoquine [12, 13].

Although bufuralol 1′-monooxygenase activity has been measured in several rat tissues, such as liver, kidney, brain, lung and spleen [14, 15], the tissue distribution of debrisoquine 4-monooxygenase activity, which is important for the metabolism of Parkinsonism-inducing substances such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and tetrahydroisoquinoline (TIQ) [14, 16, 17], has not been reported and could be different from the distribution of bufuralol 1′-monooxygenase activity.

In this study bovine and rabbit tissues were used because of their size, especially small organs such as adrenal glands and various parts of the brain which renders the preparation of microsomes, the purification and extraction of RNA, and the detection of localized proteins or mRNAs easier.

We have cloned and analysed cDNA clones of the P450 IID subfamily from bovine liver, and reported the presence of microheterogeneity§. To study the functions and expressional control of members of the P450 IID subfamily, the tissue distributions of debrisoquine 4-monooxygenase and bufuralol 1′-monooxygenase activities in bovine and rabbit tissues were examined in this study.

MATERIALS AND METHODS

Chemicals. Debrisoquine sulfate, 4-hydroxydebrisoquine sulfate, bufuralol hydrochloride (rac-α- [(*tert*- butylamino)methyl]- 7-ethyl-2-benzofuranmethanol hydrochloride) and 1′-hydroxybufuralol were kindly supplied by Dr H. Fukui (Department

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‡ Abbreviations: P450, cytochrome P450; LKM, liver kidney microsomes; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; TIQ, tetrahydroisoquinoline; G6P, D-glucose 6-phosphate; G6PDH, glucose-6-phosphate dehydrogenase; SOD, superoxide dismutase.

§ Tsuneoka Y, Matsuo Y and Ichikawa Y. Characterization and microheterogeneity of cytochrome P-450 IID subfamily from bovine liver: nucleotide sequence and classification of the members of cytochrome P-450 IID subfamily. Manuscript in preparation.

of Pharmacology, Medical School, Osaka University). D-Glucose 6-phosphate (G6P) and glucose-6-phosphate dehydrogenase (G6PDH) from *Leuconostoc mesenteroides* were purchased from Oriental Yeast Co. Ltd, Japan. NADPH and phenylmethyl sulfonyl fluoride were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Superoxide dismutase (SOD) was purchased from Toyobo Co. Ltd, Japan.

Materials. The cattle (Holstein–Friesian, 1.5 years old) were obtained from a local slaughterhouse. The rabbits (Japan, white, 6 months old) had been given a normal diet and water *ad lib.* in the laboratory. Fresh tissues from the animals were kept on ice and transported to the laboratory within 1 hr. They were perfused with ice-cold 0.15 M KCl to remove blood. They were then rapidly and carefully cleared of connective tissue, fat and major blood vessels.

Preparation of microsomes. Microsomal fractions from bovine and rabbit tissues were prepared by the method of Mitoma *et al.* [18]. Care was taken to remove hemoglobin as much as possible, as described previously [19]. Contamination of the microsomes of various tissues by mitochondria was found to be less than 5% on a protein basis, as judged from the succinate dehydrogenase activity [20].

Using bovine serum albumin as a standard, the protein concentrations in microsomal fractions were measured by the biuret reaction method [21] in the presence of 0.25% (w/v) deoxycholic acid, which was added to remove turbidity from the sample. The increased absorption at 540 nm in the biuret reaction due to heme in the test samples was avoided by using a solution containing 3.4% (w/v) NaOH, KI (1 g/500 mL) and potassium sodium tartrate 3 g/500 mL). The optical absorption at 25° was measured, using cuvettes of 1 cm optical path, with a Cary recording spectrophotometer, model 17D.

Assaying of debrisoquine 4-monooxygenase and bufuralol 1′-monooxygenase activities. The enzymatic reactions and chromatographic conditions reported by Kronbach *et al.* [10] were slightly modified and used for the activity assays. In the NADPH-regenerating system, G6P and G6PDH were used instead of sodium isocitrate and isocitrate dehydrogenase. The reaction mixture, which contained microsomal protein (0.3–2 mg), a NADPH regenerating system without NADPH (5 mM G6P, 2.5 U G6PDH and 4 mM $MgCl_2$) and 0.5 mM substrate (debrisoquine or bufuralol concentrations of more than three times the K_m values reported for human and rat P450db) in 0.05 M sodium phosphate buffer (pH 7.4), was preincubated for 3 min at 37°. In the reactions with pancreas microsomes, phenylmethyl sulfonyl fluoride was added to 0.5 mM to inhibit protease activity. The reaction was started by the addition of NADPH (final concentration, 1.6 mM), followed by aerobic incubation for 60 min at 37°. Under these conditions, the enzymatic reactions were linear with time for at least 80 min and microsomal protein concentrations up to 2 mg/mL. Only when the presence of a trace of activity was checked, the mixture was incubated for 2 hr at 37°. The final volume of the reaction mixture was 1 mL. The reaction was stopped by the addition of 100 μL of 60% $HClO_4$ (w/v). Denatured protein was removed by centrifugation. The enzymatic production of 4-hydroxydebrisoquine or 1′-hydroxybufuralol was measured by HPLC (Waters 510), with fluorescence detection, as described by Kronbach *et al.* [10]. The column used was of Cosmosil 5C-18 (Nacalai Tesque, 4.6 × 250 mm) for both activity assays. In most cases, the retention times of 1′-hydroxybufuralol and 4-hydroxydebrisoquine were 4.2 and 9.5 min, respectively. In some cases, however, overlapping peaks of products had to be separated by adjusting the retention time of 4-hydroxydebrisoquine in the range of 9.3 to 18.8 min.

In the inhibition experiment, phenylisocyanide, KCN, NaN_3, metyrapone, sodium cholate and quinidine were added to reaction mixtures to final concentrations of 0.1 mM, 1 mM, 5 mM, 1 mM, 0.5% (w/v) and 0.5 mM, respectively. Mannitol, a hydroxyl radical scavenger [22], and SOD were added to a final concentration of 200 mM and 1 μM, respectively.

RESULTS

Debrisoquine 4-monooxygenase activity in various tissues

Debrisoquine 4-monooxygenase and bufuralol 1′-monooxygenase activities in the microsomes of various tissues from male and female cattle, and male and female rabbits were analysed. The results are summarized in Table 1.

A high level of debrisoquine 4-monooxygenase activity was detected in liver from both cattle and rabbits, and a low level in the cerebral cortex of cattle, lung of cattle and rabbits, small intestine of rabbits, spleen of cattle and kidney cortex of rabbits. No significant sex differences were detected for the debrisoquine 4-monooxygenase activity in the rabbit tissues studied.

Debrisoquine 4-monooxygenase activity in male rabbit liver was eight times higher than in male bovine liver (35.5 ± 6.5 vs 4.4 ± 0.6 nmol/hr/mg protein) The order of activity in rabbit liver was the same as in rat liver [9], and that in bovine liver was the same as in human liver [10, 23].

Debrisoquine 4-monooxygenase activity in bovine cerebral cortex was about 3% of the liver value. Because of the low level, the activity could not be detected when rabbit microsomes from whole cerebrum were analysed as to enzyme activity. None of the other brain parts, i.e. midbrain, cerebellum and medulla oblongata, showed detectable activity. This suggested that debrisoquine 4-monooxygenase activity is higher in the cerebral cortex region of the cerebrum than any other brain parts studied. This is consistent with the regional distribution of spartein monooxygenase activity in the brain [24].

Debrisoquine 4-monooxygenase activity in *bovine* kidney cortex could not be detected, but low levels of activity, 1.5% and 0.8% of the liver value, were found in male and female *rabbit* kidney cortex, respectively.

In lung, activity levels of 0.7% and 0.5% of the liver value were observed in male cattle and female rabbits, respectively. The activities in small intestine, 0.3% of the liver value, and spleen, 0.6% of the

Table 1. Debrisoquine 4-monooxygenase and bufuralol 1′-monooxygenase activities in microsomes of various tissues from bovine and rabbit

Tissues	Animals	Sex	Debrisoquine 4-monooxygenase (nmol/hr/mg protein)	(%)	Bufuralol 1′-monooxygenase (nmol/hr/mg protein)	(%)
Liver	Bovine	Male	4.417 ± 0.551(2)	100	34.397 ± 4.318(2)	100
	Rabbit	Male	35.526 ± 6.491(2)	100	66.596	100
	Rabbit	Female	23.812	100	65.729	100
Kidney						
Cortex	Bovine	Male	ND(3)		0.392 ± 0.328(3)	1.14
	Rabbit	Male	0.526	1.48	1.653 ± 1.955(5)	2.48
	Rabbit	Female	0.187	0.79	2.933 ± 2.007(3)	4.46
Medulla	Rabbit	Male	ND(2)		0.540 ± 0.592(2)	0.82
	Rabbit	Female	ND(2)		0.144 ± 0.172(2)	0.22
Adrenal gland						
Cortex	Bovine	Male	ND(3)		0.133 ± 0.035(3)	0.39
Medulla	Bovine	Male	ND(2)		0.075 ± 0.006(2)	0.22
Brain						
Cerebral cortex	Bovine	Male	0.132 ± 0.083(3)	2.99	0.085 ± 0.050(3)	0.25
Cerebrum	Rabbit	Male	ND		0.018	0.03
	Rabbit	Female	ND		0.026	0.04
Midbrain	Rabbit	Male	ND		0.017	0.03
Cerebellum	Rabbit	Male	ND		0.010	0.02
	Rabbit	Female	ND		0.009	0.01
Medulla oblongata	Rabbit	Male	ND		0.010	0.02
Lung	Bovine	Male	0.032	0.72	0.196	0.57
	Rabbit	Female	0.127	0.53	0.843	1.28
Small intestine	Bovine	Male	ND		0.024	0.07
	Rabbit	Female	0.070	0.29	0.076	0.12
Blood vessels	Bovine	Male	ND		0.005	0.01
Thyroid gland	Rabbit	Male	ND		0.053	0.08
	Rabbit	Female	ND		0.063	0.10
Heart	Rabbit	Female	ND		0.038	0.06
Pancreas	Bovine	Male	ND		0.022	0.06
	Rabbit	Female	ND		0.039	0.06
Spleen	Bovine	Male	0.028	0.63	0.067	0.19
	Rabbit	Female	ND		0.016	0.02
Ovary	Bovine	Female	ND		0.019	
	Rabbit	Female	ND		0.017	0.03
Uterus	Rabbit	Female	ND		0.034	0.05
Testis	Rabbit	Male	ND		0.165	0.25

Values are means ± SD. Within parentheses are the number of samples from different animals.

ND, not detected; the enzyme activities less than 0.025 and 0.001 nmol/hr/mg protein of microsomes cannot be detected for debrisoquine 4-monooxygenase and bufuralol 1′-monooxygenase, respectively.

liver value, were detected in only one of the two species studied, respectively.

There was no detectable debrisoquine 4-monooxygenase activity in kidney medulla, adrenocortex, adrenomedulla, blood vessels, thyroid gland, heart, pancreas, ovary, uterus or testis. To check for the presence of a trace of debrisoquine 4-monooxygenase activity in bovine kidney cortex and adrenocortex, the reaction mixtures were incubated longer (2 hr). However, 4-hydroxydebrisoquine, the product of the reaction, could not be detected in these tissues. Low levels of other unknown metabolites were detected in microsomes of the adrenocortex (Fig. 1).

Bufuralol 1′-monooxygenase activity in various tissues

Bufuralol 1′-monooxygenase activities in various tissues are shown in Table 1 and representative chromatographic patterns in Fig. 2. There was a general tendency that the higher was the debrisoquine 4-monooxygenase activity in certain tissues, the higher was the bufuralol 1′-monooxygenase activity in the same tissue. High levels of bufuralol 1′-monooxygenase activity were found in liver, and low levels in kidney cortex, adrenocortex, adrenomedulla, cerebral cortex, lung, small intestine and spleen. Traces of activity were found in cerebrum, midbrain, cerebellum and medulla oblongata from rabbit brain, and blood vessels, thyroid gland, heart, pancreas, ovary, uterus and testis.

The activity levels in liver from male cattle, and male and female rabbits were 34.4, 66.6 and 65.7 nmol/hr/mg protein, respectively. The bufuralol 1′-monooxygenase activity levels in kidney cortex from male cattle, and male and female rabbits were 1.1%, 2.5% and 4.5% of the liver values, respectively.

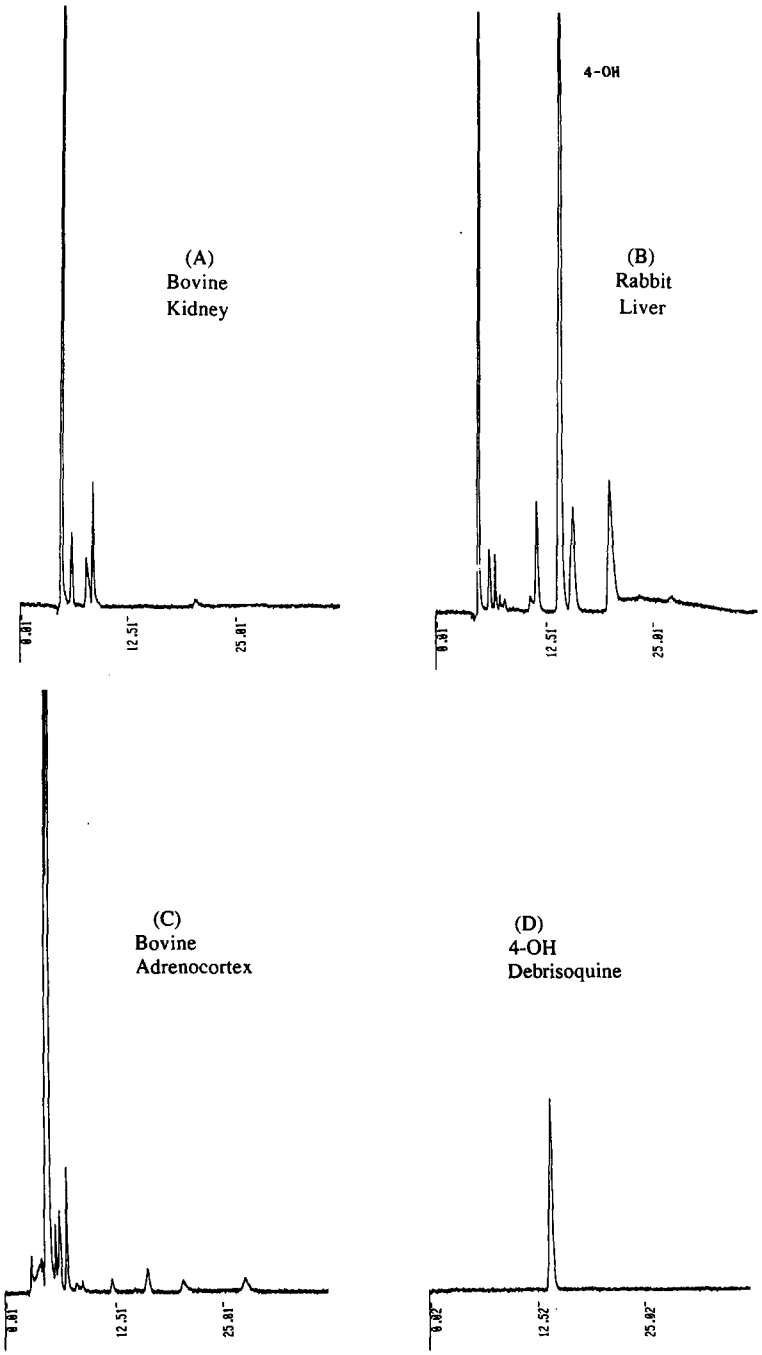

Fig. 1. Chromatograms of debrisoquine metabolites with a longer incubation period (2 hr). (A) Incubation of kidney cortex microsomes from male cattle with debrisoquine. (B) Liver microsomes from male rabbits. 4-OH, 4-hydroxydebrisoquine. (C) Adrenocortex microsomes from male cattle. (D) Standard 10.4 nmol of 4-hydroxydebrisoquine. Six hundred microlitres of the reaction mixture were injected in each case.

Bufuralol 1′-monooxygenase activity was found in adrenal cortex and medulla, 0.4% and 0.2% of the liver value, respectively. In adrenal cortex and medulla, no debrisoquine 4-monooxygenase activity was detected, suggesting the presence of db2 type P450 in adrenal cortex and medulla.

A low level of bufuralol 1′-monooxygenase activity was found in cerebral cortex (0.3% of the liver value) and very low levels in other brain parts. Like debrisoquine 4-monooxygenase activity in cerebral cortex, bufuralol 1′-monooxygenase activity was also higher in the cerebral cortex region of the brain.

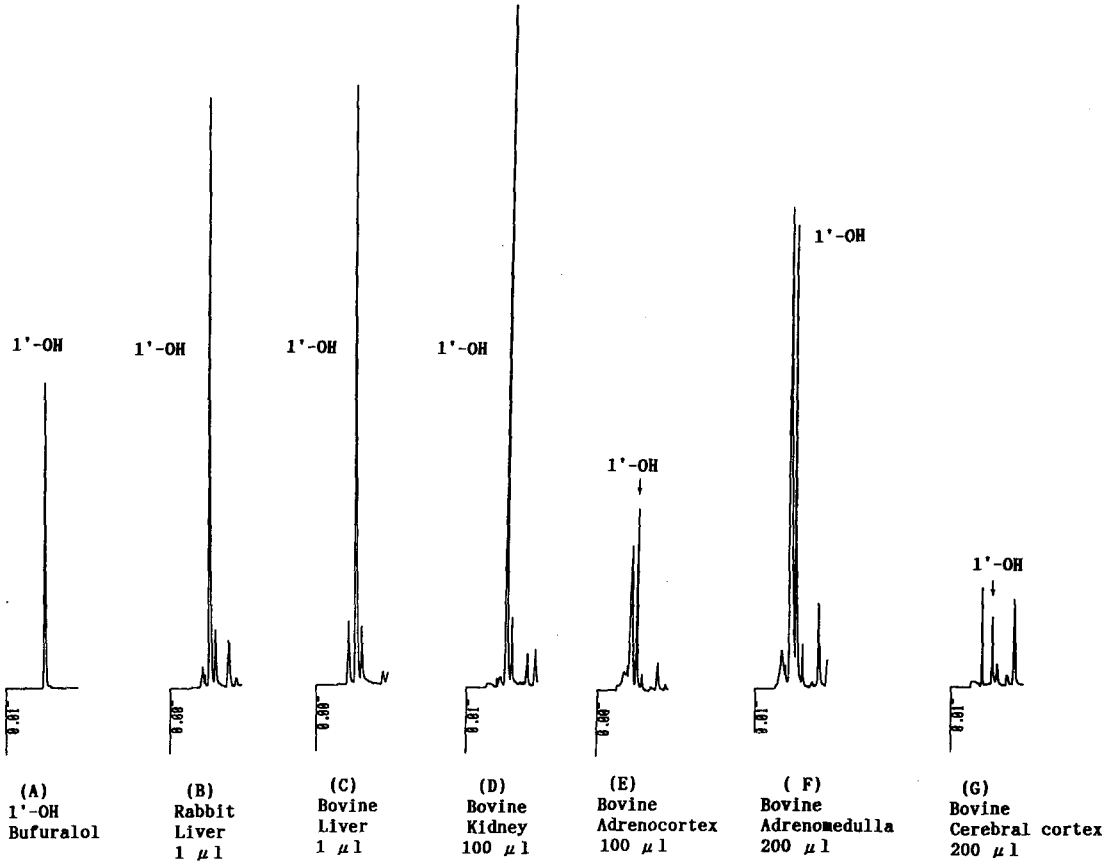

Fig. 2. Chromatograms of bufuralol metabolites. (A) Forty picomoles of 1′-hydroxybufuralol as a standard. (B) Metabolites formed by liver microsomes from male rabbits. One microlitre of the reaction mixture was injected. 1′-OH, 1′-hydroxybufuralol. (C) Liver microsomes from male cattle, 1 μL. (D) Kidney cortex microsomes from male cattle, 100 μL. (E) Adrenocortex microsomes from male cattle, 100 μL. (F) Adrenomedulla microsomes from male cattle, 200 μL. (G) Cerebral cortex microsomes from male cattle, 200 μL.

Other tissues which showed low levels of bufuralol 1′-monooxygenase activity were lung, small intestine and spleen. These tissues also showed low levels of debrisoquine 4-monooxygenase activity. No significant sex difference was detected for bufuralol 1′-monooxygenase activity in rabbits.

Bufuralol/debrisoquine monooxygenase ratio in various tissues

The bufuralol 1′-monooxygenase/debrisoquine 4-monooxygenase activity ratios are shown in Table 2. There was variation in the ratio in various tissues. In liver, the ratios were 7.8 in male cattle, 1.9 in male rabbit and 2.8 in female rabbit, however, the ratios in kidney cortex were 3.1 in male rabbit and 15.7 in female rabbit. These values were higher than in liver. In lung the ratio in female rabbit, 6.6, was higher than in liver, but the ratio in male cattle, 6.1, was about the same as in liver. In contrast, bovine cerebral cortex and spleen showed lower ratios, 0.6 and 2.4, respectively, than liver.

Effects of inhibitors on debrisoquine 4-monooxygenase and bufuralol 1′-monooxygenase activities

To confirm the P450 dependent reaction, the dependence of the reaction on NADPH as an electron donor, the effects of six chemicals, phenylisocyanide, KCN, NaN_3, metyrapone, sodium cholate and quinidine, on the debrisoquine 4-monooxygenase and bufuralol 1′-monooxygenase activities were investigated with microsomes from several bovine tissues, i.e. liver, kidney cortex, adrenocortex, adrenomedulla and cerebral cortex. Phenylisocyanide is known to be a specific inhibitor of P450 [25] and sodium cholate is a denaturing reagent, converting P450 to P420 [26]. The results are shown in Table 3.

Debrisoquine 4-monooxygenase activity in liver was strongly inhibited by 0.1 mM phenylisocyanide, 5 mM NaN_3 and 0.5 mM quinidine to 1.6%, 9.4% and 8.7% of the control value, respectively, and moderately by 1 mM metyrapone and 0.5% (w/v) sodium cholate, to 16.3% and 23.2%, respectively,

Table 2. Ratios of bufuralol 1′-monooxygenase (Buf) to debrisoquine 4-monooxygenase (Deb) activities in various tissues

Tissues	Animals	Sex	Ratios (Buf/Deb)
Liver	Bovine	Male	7.79
	Rabbit	Male	1.87
	Rabbit	Female	2.76
Kidney cortex	Rabbit	Male	3.14
	Rabbit	Female	15.68
Brain cerebral cortex	Bovine	Male	0.64
Lung	Bovine	Male	6.13
	Rabbit	Female	6.64
Small intestine	Rabbit	Female	1.09
Spleen	Bovine	Male	2.39

but not inhibited by 1 mM KCN. The reaction without NADPH or a substrate showed no detectable debrisoquine 4-monooxygenase activity. A similar activity inhibition pattern was seen in cerebral cortex. Because of the low activity in cerebral cortex, which was just above background, the activity inhibited by phenylisocyanide, NaN_3, metyrapone and sodium cholate could not be detected. The activity was not inhibited by KCN, 90.5%, and not detected without NADPH or a substrate.

Bufuralol 1′-monooxygenase activities in microsomes from the various tissues were inhibited very similarly by these inhibitors, except for cerebral cortex. The activities of various tissues were inhibited strongly by 0.1 mM phenylisocyanide, 0.5% (w/v) sodium cholate and 0.5 mM quinidine, 4–18% of the control value, and moderately by 1 mM metyrapone and 5 mM NaN_3, 20–69%. One millimolar KCN did not inhibit the activity, 93–104%. No detectable activity was found without a substrate. However, traces of activity were detected without NADPH, 0.003–0.055 nmol/hr/mg protein, in which case the reaction might be driven by either endogenous NADPH or an enzyme that is independent of NADPH. The activity inhibition pattern in cerebral cortex seemed to be different. However, the activity was inhibited 49.1%, 32.1% and 39.6% by 0.1 mM phenylisocyanide, 5 mM NaN_2 and 0.5% sodium cholate, respectively, but not at all by 1 mM KCN or 1 mM metyrapone.

These results of inhibition experiments, i.e. that the activities in several tissues were strongly inhibited by phenylisocyanide, inhibited very similarly by several inhibitors and depended on NADPH, confirmed that the low level of the debrisoquine 4-monooxygenase reaction in brain, and the bufuralol 1′-monooxygenase reactions detected in kidney cortex, adrenal gland and brain, as well as the high level in liver, were catalysed by P450.

P450s have been classified into two groups according to their sensitivity to KCN [27, 28]. From the results of the inhibition experiments, it is clear that P450 db belongs to the group insensitive to KCN.

Effects of a hydroxyl radical scavenger and SOD on the enzyme activities

To investigate the effects of hydroxyl radicals and O_2^-, a hydroxyl radical scavenger, mannitol, and SOD were added to final concentrations of 200 mM and 1 μM, respectively, and then the enzyme activities in several bovine tissues were measured (Table 3). Although debrisoquine 4-monooxygenase activity in liver in the presence of mannitol was decreased to 70%, bufuralol 1′-monooxygenase activity did not change, 82–107% of the control value. SOD did not affect the enzyme activities, 97–114% of the control values, in the tissues studied. These results show that neither hydroxyl radicals nor O_2^- had significant effects on these activity assays.

DISCUSSION

Bufuralol 1′-monooxygenase activity has been reported in several rat tissues, such as liver, kidney, brain, adrenal, lung and spleen [15]. The activities were very close to those reported here for cattle and rabbits. Debrisoquine 4-monooxygenase activity in various tissues was also determined and variations in the bufuralol/debrisoquine monooxygenase activity ratio were found. Moreover, the ratios in a single tissue in two species were also different, for example, the level of debrisoquine 4-monooxygenase activity in rabbit liver was eight times higher than that in bovine liver. However, bufuralol 1′-monooxygenase activity was not very different in the livers of the two species. The variation in the activity ratio in tissues or species could be explained by either or both of the following. (1) The efficiency of debrisoquine or bufuralol metabolism by a single P450 molecule. (2) The ratio of the expressed proteins, db1 type and db2 type. To clarify this, enzymatic and kinetic parameters, such as K_m and V_{max}, and the turnover numbers for each type of db protein and the degree of expression of each member of the subfamily in tissues should be studied.

Drug metabolizing activities, such as those of 7-benzoxyresorufin *O*-dealkylase, 7-pentoxyresorufin *O*-dealkylase, 7-ethoxyresorufin *O*-deethylase, 7-ethoxycoumarin *O*-deethylase, aryl hydrocarbon hydroxylase [29, 30], bufuralol 1′-monooxygenase [14, 16] and spartein monooxygenase [24], have been found in brain. In this study, low levels of the activities of both debrisoquine 4-monooxygenase and bufuralol 1′-monooxygenase were found in cerebral cortex. The presence of P450 db in cerebral cortex is consistent with Western blotting data [31]. The finding of the presence of debrisoquine/spartein monooxygenase activities in brain is important for understanding the metabolism of Parkinsonism-inducing substances such as MPTP and TIQ. These neurotoxins or chemicals can be metabolized not only in liver but also in brain.

Low levels of bufuralol 1′-monooxygenase and debrisoquine 4-monooxygenase activities, with a higher ratio, were detected in kidney cortex. This is consistent with the following observations. Firstly, serum from LKM1 patients, who possess auto-antibodies for P450 db1 (IID6) [32–34], cross-reacts

Table 3. Effects of inhibitors, hydroxyl radical scavenger and SOD on the enzyme activities of debrisoquine 4-monooxygenase and bufuralol 1'-monooxygenase in microsomes of various tissues from male cattle

	Debrisoquine 4-monooxygenase		Bufuralol 1'-monooxygenase				
	Liver	Cerebral cortex	Liver	Kidney cortex	Adrenal Cortex	Adrenal Medulla	Cerebral cortex
Control	4.806 ± 0.446*[4]	0.084	31.343 ± 3.000[4]	0.203 ± 0.022[2]	0.113 ± 0.011[2]	0.071 ± 0.014[2]	0.053
	(100)	(100)	(100)	(100)	(100)	(100)	(100)
Phenylisocyanide	0.075		4.884	0.035	0.016	0.005	0.027
(0.1 mM)	(1.6)	ND**	(15.6)	(17.2)	(14.2)	(7.0)	(50.9)
KCN	5.522	0.076	29.205	0.192	0.118	0.070	0.061
(1 mM)	(114.9)	(90.5)	(93.2)	(94.6)	(104.4)	(98.6)	(115.1)
NaN_3	0.454		13.019	0.140	0.072	0.022	0.036
(5 mM)	(9.4)	ND	(41.5)	(69.0)	(63.7)	(31.0)	(67.9)
Metyrapone	0.785		6.312	0.075	0.028	0.022	0.080
(1 mM)	(16.3)	ND	(20.1)	(36.9)	(24.8)	(31.0)	(150.9)
Sodium cholate	1.114		2.463	0.009	0.008	0.004	0.032
(0.5%)	(23.2)	ND	(7.9)	(4.4)	(7.1)	(5.6)	(60.4)
Quinidine	0.417 ± 0.389 [2]		5.737 ± 0.158 [2]				
(0.5 mM)	(8.7)	—	(18.3)	ND	ND	ND	—
Mannitol	3.364 ± 0.771 [2]		33.216 ± 0.905 [2]	0.168	0.103	0.076	
(200 mM)	(70.0)	—	(106.0)	(82.8)	(91.2)	(107.0)	—
SOD	5.116		35.734	0.215	0.122	0.069	
(1 μM)	(106.5)	—	(114.0)	(106.0)	(107.6)	(96.9)	—

* Enzyme activities are expressed as nmol/hr/mg protein (mean ± SD). The number of samples is indicated in brackets. Within round brackets are the percentage activities relative to control value.

ND, not detected; the enzyme activities less than 0.025 and 0.001 nmol/hr/mg protein of microsomes cannot be detected for debrisoquine 4-monooxygenase and bufuralol 1'-monooxygenase, respectively.

— Effects of quinidine, mannitol and SOD on enzyme activities of cerebral cortex have not been studied.

with microsomal protein in kidney cells as well as the high levels in liver cells [35, 36]. Secondly, in rat kidney, mRNAs for IID2 and IID3 were detected in considerably larger amounts than those of IID1 (which exhibits debrisoquine 4-monooxygenase activity) and IID5 [37]. Therefore, the higher bufuralol 1′-monooxygenase activity ratio in kidney cortex can be explained by a higher ratio of the db2 type protein.

In lung, low levels of debrisoquine 4-monooxygenase and bufuralol monooxygenase activities were detected. However, none of the rat P450 IID forms were detected in intestine or lung [11]. This might be either due to the species difference or to the different sensitivities of the methods used. The role of P450 db1 in lung might be related to susceptibility to lung cancer. A positive relationship between lung cancer and extensive metabolism of debrisoquine has been reported [13]. The physiological roles of debrisoquine 4-monooxygenase and bufuralol 1′-monooxygenase activities in small intestine and spleen are not clear, however, they are most likely involved in drug metabolism.

In the adrenocortex, some drug metabolizing activities, such as those of benzphetamine *N*-demethylase [38], ethylmorphine demethylase [39], benzo(*a*)pyrene hydroxylase [39–42] and aryl hydrocarbon hydroxylase [43], have been detected, including P450s, i.e. IA1, IIA1, IIB1, IIC7 and IIIA1, by Western blotting [44]. The presence of mRNAs for the P450 IID subfamily in the adrenocortex was confirmed by means of *in situ* hybridization and by amplifying cDNA by polymerase chain reaction [45].

No detectable P450 activity has been reported in the adrenomedulla of cattle [46] but we detected a low level of the bufuralol 1′-monooxygenase reaction, which was catalysed by P450. This enzymatic activity was not due to contamination by adrenocortex, because the low level of contamination, which if any, is several per cent at the maximum, cannot explain the difference in bufuralol 1′-monooxygenase activity between adrenal cortex and medulla. Moreover, the signal obtained on *in situ* hybridization with a P450IID cDNA probe in the boundary regions of the adrenal cortex and medulla showed approximately the same ratio for bufuralol activity [45]. This is the first evidence for P450 catalysing activity in microsomes from adrenomedulla.

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