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PRELIMINARY COMMUNICATIONS

7-ETHOXYCOUMARIN O-DE-ETHYLASE ACTIVITY IN RAT BRAIN MICROSOMES

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INTRODUCTION

Brain now appears to be susceptible to the action of xenobiotics including mutagens and carcinogens (1-3). Studies of Cohn et al., (4) and ours (5,6) have demonstrated the presence of cytochrome P-450 and aryl hydrocarbon hydroxylase in rat brain. N-demethylation of aminopyrine meperidine (7) and morphine (8), desulfuration of parathion to paraoxon (9), and hydroxylation of estrogens to 2-hydroxyestrogens (10) have also been reported to occur in rat brain. A correlation between mestranol-induced hypertension and brain contents of cytochrome P-450 and catechol estrogen formation has been suggested for rat brain (11). The comparison of properties of brain aryl hydrocarbon hydroxylase (5,6,12) and N-demethylation of aminopyrine and meperidine (7) have suggested certain similarities and divergencies from those of liver. 7-ethoxycoumarin is a model monooxygenase substrate which is metabolised by a number of different forms of hepatic and extra hepatic cytochrome P-450. In the present communication we demonstrate the presence of 7-ethoxycoumarin O-de-ethylase activity in rat brain microsomes.

MATERIALS AND METHODS

Animals: Male Wistar albino rats (90 ± 15g) derived from Industrial Toxicology Research Centre animal breeding colony raised on commercial pellet diet (Hind, Lever, Bombay) and water ad libitum were used in the experiments.

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Table 1

Cofactor Requirements of 7-Ethoxycoumarin O-De-Ethylase Activity in Rat Brain and Liver Microsomes

Conditions	p moles 7-hydroxycoumarin formed per minute per mg protein	
	BRAIN	LIVER
Complete System*	1.6	353
Complete System (boiled enzyme)	<0.02	<0.02
Complete System - NADPH - NADH	<0.02	16
Complete System - NADPH + NADH	0.6	132
Complete System + NADPH - NADH	1.3	299

Whole brain microsomal protein (1.5 mg) or liver microsomes (0.38 mg) were incubated at 37°C with the components of assay system (1.0 μmole of NADPH and 1.0 μmole of NADH). This represents the complete system. Other additions or deletions were done as indicated. Data from a typical experiment repeated several times with similar values is presented.

Table 2

Effect of Phenobarbital and 3-Methylcholanthrene Administration to Rats on Brain and Liver 7-Ethoxycoumarin O-De-Ethylase Activity

Treatment	pmoles 7-hydroxycoumarin/minute/mg protein			
	BRAIN	Percent Increase	LIVER	Percent Increase
Control*	2.2 ± 0.2+	-	308 ± 30‡	-
Phenobarbital	3.0 ± 0.1‡	36	595 ± 20‡	57
3-Methylcholanthrene	4.2 ± 0.4‡	91	2024 ± 35‡	433

*Saline- and ground nut oil-treated values did not differ and therefore they have been grouped together.

+Mean ± S.E. of four animals.

‡Statistically significant from controls ($p < 0.05$).

Preparation of Microsomes: The animals were killed by cervical dislocation. Brain and liver were removed and washed with chilled saline to remove blood clots. Brain and liver microsomes were prepared as described earlier (5,6). Essentially the tissues were homogenized in 4-volumes of ice-cold 0.1M phosphate buffer, pH 7.4, containing 0.15M KCl. The liver homogenates were centrifuged at 9000 xg for 20 minutes at 0°C whereas brain homogenates were centrifuged at 14000 xg for 20 minutes. The resulting tissue supernatants were subsequently centrifuged at 104,000 xg for 1 hour in a MSE superspeed ultracentrifuge to obtain microsomes. Microsomes were suspended in homogenizing media and used as the enzyme source.

Induction Experiment: Rats were given intraperitoneal injections daily for three days of either phenobarbital (80 mg/kg body wt. in 0.2 ml of saline) or 3-methylcholanthrene (30 mg/kg body wt. in 0.2 ml of ground nut oil). The control animals received an identical volume of the vehicle. Animals were sacrified 24 hours after the last injection. In order to avoid any possible diurnal variations all treatments and killing were done between 9-11 A.M.

Enzyme Assay: 7-ethoxycoumarin O-de-ethylase activity in brain microsomes was determined after a slight modification of the procedure of Greenlee and Poland (13). The reaction mixture in a total volume of 1.0 ml contained 65.0 μmol of potassium phosphate buffer (pH 7.2), 1.0 μmol of NADPH, 1.0 μmol of NADH, 5.0 μmol of $MgCl_2$, 1.0 mg of bovine serum albumin and 2.0 μmol of 7-ethoxycoumarin (in 50 μl of methanol). The nucleotide concentrations employed were optimal for brain enzyme activities. After 15 and 30 minutes of incubation for liver or brain enzyme, respectively, at 37°C in air the reaction was terminated by adding 0.125 ml of 15% (w/v) trichloroacetic acid. 7-Hydroxycoumarin formed was extracted into 2.0 ml of chloroform. One ml of the organic phase was extracted with 2.0ml of 0.01N NaOH-1M NaCl. Fluorescence of 7-hydroxycoumarin formed was measured at excitation and emission wavelengths of 368 and 456 nm, respectively, in the Aminco SPF-500 spectrofluorometer. The amount of 7-hydroxycoumarin formed is calculated in comparison to an authentic standard of 7-hydroxycoumarin and specific enzyme activity is expressed as pmol of product formed per minute per mg of protein.

Protein Determination: Protein was estimated according to Lowry et al., (14) using bovine serum albumin as the reference standard.

RESULTS AND DISCUSSION

The results summerised in Table 1 demonstrate the presence of 7-ethoxycoumarin O-de-ethylase activity in microsomes prepared from the brain of albino rats. The enzyme activity in brain microsomes was NADPH-dependent. The activity in the presence of NADH (1.0 μmol) alone was only 46% that of NADPH. Addition of NADH to the incubation mixture containing NADPH resulted in a 23% increase in 7-hydroxycoumarin formation. Since this increase was appreciable we decided to add NADH to routine incubation systems. No 7-hydroxycoumarin formation was noticed when either boiled enzyme was used in the complete incubation system or pyridine nucleotides were omitted from the reaction mixture. The level of microsomal ethoxycoumarin O-de-ethylase in rat brain is approximately 0.5% of the corresponding liver values.

The effect of phenobarbital and 3-methylcholanthrene treatments on microsomal 7-ethoxycoumarin O-de-ethylase activity in rat brain is shown in Table 2. Phenobarbital treatement to rats increased the level of 7-ethoxycoumarin O-de-ethylase in liver by 57% and in brain by 36%. The treatment with 3-methylcholanthrene resulted in 433 and 91% induction in liver and brain enzyme, respectively. It is interesting to note that both brain and liver 7-ethoxycoumarin O-de-ethylase were more sensitive to the effect of 3-methylcholanthrene than that of phenobarbital indicating similarities in enzyme induction in these two tissues.

The data presented clearly shows that brain does contain inducible 7-ethoxycoumarin O-de-ethylase activity. The data further emphasizes that brain microsomes are capable of metabolising a wider class of mixed function oxidase substrates. Studies are underway to better define the range of substrates metabolised by the brain monooxygenase system. Since brain microsomes are capable of metabolising a range of substrates it is possible that multiple forms of cytochrome P-450 exist in brain microsomes.

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