COMPARATIVE STUDIES OF HEPATIC NICOTINE METABOLIZING ENZYME ACTIVITIES IN MONKEYS AND DOGS

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It has been demonstrated that the Rhesus monkey was 5 to 10 times less sensitive than the dog regarding the effects of nicotine on gastrointestinal contractility (1). While, in studies of biological fate of ^3H -nicotine, the nicotine concentrations in CNS and liver in monkeys were found to be much lower than those in dogs (2). The time in which ^3H -nicotine: total ^3H ratio became 0.5 in plasma after i.v. injection of ^3H -nicotine was very short in both species; in addition, that in monkeys was short as compared to dogs (3). These results suggest that there may be a difference between the two species in the rate of nicotine destruction, even though nicotine was destroyed very rapidly in both species. This paper reports studies of microsomal drug metabolizing enzyme activities in the liver of the two species.

Rhesus monkeys and dogs were anesthetized with a rapid i.v. injection of 150 mg/kg of MgSO_4 and some dogs were sacrificed by a blow on the head. Animals were sacrificed around 10:00 a.m. by immediate exsanguination via the carotid artery. The liver was homogenized with 3 volumes of 1.15% KCl solution in a Potter-Elvehjem's teflon-glass homogenizer. A liver supernatant fraction was prepared by centrifugation of the homogenate at $10,700 \times g$ for 40 min. This supernatant fraction was centrifuged at $105,000 \times g$ for 1 hr and the firmly packed pellet of microsomes was re-suspended in 1.15% KCl solution with the Potter-type homogenizer and again centrifuged as above. Finally, the washed microsomal pellet was suspended in 1.15% KCl solution containing 50 mM Tris-HCl buffer, pH 7.4 and diluted to a concentration of 2 mg protein per ml. The supernatant fraction, 2.5 ml, was mixed with a solution containing 1.5 μmole NADP, 25 μmole G-6-P, 25 μmole MgCl_2 and 3 μmole ^3H -nicotine. The final volume made a total of 5.0 ml with 0.1 M sodium, potassium phosphate buffer, pH 7.4.

It was previously reported that nicotine metabolism by rabbit liver microsomes was affected by the addition of nicotinamide (4). In preliminary experiments, nicotine metabolizing enzyme activities in the liver of monkeys were reduced approx. 10% by the addition of 50 μmole of nicotinamide (final concentration: 10^{-3} M) and approx. 5% in the liver of dogs. The experiments described in this report were performed using a nicotinamide-free media.

Metabolism of nicotine was determined by measuring the radioactivity of ^3H -nicotine remaining, in a liquid scintillation spectrometer (Packard Model 3320) by the method

reported by Hug (5) and described in details by Tsujimoto et al. (3). The amount of cytochrome P-450 was determined by measuring the CO difference spectra of dithionite-reduced samples according to the method of Omura and Sato (6) using a Hitachi Model 356 spectrophotometer. The changes in optical density (ΔOD values) were determined from the difference spectra caused by the addition of nicotine to the microsomal suspension according to the method of Schenkman et al. (7). Protein concentrations of the liver supernatant fraction and the microsomal suspension were estimated by the method of Lowry et al. (8) using bovine serum albumin as the standard.

As shown in Table 1, nicotine metabolizing enzyme activities in the liver of monkeys were found to be significantly greater than those in the liver of dogs. Double-reciprocal plots of nicotine metabolizing enzyme activities in the liver of monkeys and dogs are shown in Fig. 1-A. The Michaelis constants (K_m) for nicotine, obtained theoretically using the least squares method were much lower in monkeys than in dogs (7.3×10^{-4} M for monkeys and 14.0×10^{-4} M for dogs respectively). The contents of cytochrome P-450 and b_5 in

TABLE 1. Comparison of nicotine metabolizing enzyme activities and heme components in the liver of monkeys and dogs.

	Monkey	Dog
Specific activity†	40.3 $\pm$ 5.5	28.2 $\pm$ 0.9
Cytochrome P-450‡	1.42 $\pm$ 0.11	0.67 $\pm$ 0.07
Cytochrome b_5 §	0.64 $\pm$ 0.07	0.41 $\pm$ 0.02
AOD§/P-450	0.027	0.028

Values represent the mean $\pm$ SE of 4 and 6 experimental values for the monkey and dog respectively.

† μ moles/g Protein/hr

‡ m μ moles/mg Protein

§ Absorbance at 430 nm relative to 395 nm caused by the addition of 5 mM of nicotine to the microsomal suspension.

FIG 1-A (left). Double-reciprocal plots of nicotine metabolizing enzyme activities in monkey and dog liver homogenates. The initial velocity is represented by dpm of ^3H -nicotine metabolized per gram protein during 10 min incubation. 1-B (right). Double-reciprocal plots of changes in absorbance at 430 nm relative to 395 nm caused by consecutive additions of nicotine to suspensions of monkey and dog liver microsomes.

monkey liver microsomes were about 2 and 1.5 times higher respectively than those in dogs (Table 1). The P-450 value in monkeys is higher than the value described by Gram et al. (9) and the value in dogs is in close agreement with the data of Cumming et al. (10).

It has been reported that the addition of substrates to liver microsomal suspension caused two types of spectral changes. One class of spectral change is characterized by the appearance of a trough at about 390 nm and an absorption peak at about 430 nm. Nicotine is included in this class (type II) (7). Microsomal cytochrome P-450 plays a role as the oxygen activating enzyme for the oxidation of a variety of drugs (11). The pigment with which these spectral changes involve interaction was shown to be cytochrome P-450 and the magnitude of the spectral changes depended on the amount of substrates and microsomal pigments added, suggesting substrate affinity to the enzyme (12). The magnitude of spectral changes caused by adding 5 mM of nicotine to monkey liver microsomal suspension was much greater than that of dog liver microsomes. The values of $\Delta OD/mg$ protein/ml for monkeys and dogs were 0.043 and 0.026 respectively. When expressed on a per μ mole cytochrome P-450 basis, the magnitude of interaction between nicotine and microsomal cytochrome P-450 isolated from the monkey liver was approx. equivalent to that seen with microsomes from the dog liver (Table 1). Figure-1-B shows double-reciprocal plots of the spectral changes caused by the addition of nicotine to the microsomal suspensions from monkey and dog livers. The spectral dissociation constants (K_s) were 3.9×10^{-4} M and 3.0×10^{-4} M for monkeys and dogs respectively and this difference in K_s value was not significant ($p > 0.2$). These results indicate that the affinity of nicotine for cytochrome P-450 in liver microsomes does not differ between monkeys and dogs. It has been reported that the activity of the liver microsomal drug oxidizing enzyme system is parallel to the cytochrome P-450 content (13, 14). Schenckman et al. (12) described that sex difference in drug metabolism can be explained as due to the difference in affinity of drugs for the terminal oxidase cytochrome P-450 and to extent of binding of substrate by these enzymes. From the results described herein, it is suggested that the greater amount of microsomal cytochrome P-450 in the liver of monkeys may contribute to the higher enzyme activities as compared to dogs.

Data in the present studies is in agreement with the in vivo results reported previously that the rate of nicotine destruction in monkeys was much faster than in dogs (3). It is considered that the higher activities of nicotine metabolizing enzymes in the monkey liver may partially result in the quantitative differences in responses to nicotine between the two species.

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CHANGE IN CATECHOLAMINE CONTENT OF *TETRAHYMENA PYRIFORMIS* W DURING GROWTH

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In 1966, Janakidevi *et al.* (1) discovered that the ciliated protozoan, *Tetrahymena pyriformis* contained catecholamines (CA) and readily converted radioactive tyrosine, phenylalanine and dihydroxyphenylalanine to CA. In our laboratory it was discovered that this protozoan contained the system catalysing the oxidation of biogenic monoamines and diamine (2). It was also found that in cultures of *Tetrahymena* monoamine oxidase activity decreased in the exponential growth phase (EGP) and increased in the stationary growth phase (SGP) (3). The present work was on the change of the endogenous CA level and the rate of conversion of tyrosine to CA during growth of this protozoan.

Tetrahymena pyriformis W was cultured as reported previously (3). After culture for various periods, cells were treated with 0.4 N perchloric acid following the procedure of Janakidevi *et al.* (1). CA was isolated by adsorption onto alumina (active neutral, Woelm) (4) and was measured fluorometrically, using adrenaline as a standard (5). CA levels during growth of cultures are expressed as total CA.

To measure the rate of conversion of tyrosine into CA, 50 m μ Ci/ml of tyrosine-¹⁴C (405 mCi/mM, Daiichi Pure Chemicals) were added to cultures at various stages of growth. After further incubation for 4 hr, radioactive CA was extracted as described above. An aliquot was dissolved in Bray's solution (6) and radioactivity was measured in a liquid scintillation counter.

The CA contents at various times during growth are summarized in Table I. The content in the early EGP was 0.39 μ g/10⁸ cells, which is in good agreement with the value of Blum *et al.* (7). The content in the SGP decreased to about one twentieth that in the EGP, therefore the endogenous CA content was higher in rapidly growing *Tetrahymena*