THE INTERACTION OF VARIOUS *N*-SUBSTITUTED AMPHETAMINES WITH CYTOCHROME *P*-450 OF RABBIT LIVER MICROSOMES

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1. Introduction

In the course of the monooxygenase reaction, there occurs a binding of substrate to oxidized cytochrome *P*-450 which is often observable in the difference spectrum of the microsomes as a type I spectral change [1, 2]. This is due to a substrate-induced shift in the absorbance in the Soret region of oxidized cytochrome *P*-450 from about 420 nm to about 390 nm [3] and is accordingly observed as a peak at about 390 nm and a trough at about 420 nm in the microsomal difference spectrum. Whereas the type I spectral change was early associated with metabolism [1], there is as yet no evidence that the type II spectral change – characterized by a trough near 390 nm and a peak between 425 and 435 nm in the microsomal difference spectrum [1, 2] – reflects the formation of a metabolically active cytochrome *P*-450–substrate complex [4], although some of the basic amines that produce the type II spectral change are also substrates of the cytochrome *P*-450 containing monooxygenase system. On the contrary, the type II spectral change is presumably due to ferrihemochrome formation caused by the direct interaction between a basic nitrogen and the heme iron [1, 2].

One of the characteristics of the cytochrome *P*-450 containing monooxygenase system of liver microsomes is its apparent lack of substrate specificity. Thus, its substrates constitute a chemically and pharmacologically quite heterogeneous group, although they seem to share one property – a certain degree of lipid solubility. Rough correlations have also been reported between lipid solubility and metabolism by the cytochrome *P*-450 system for various groups of compounds [5–7]. On the other hand, in a recent study on the interaction of a series of barbiturates with cytochrome *P*-450, it was concluded that although lipid solubility is required for a substance to reach microsomal cytochrome *P*-450, other properties of the molecule are of importance for determining the affinity with which it will interact with the cytochrome [8].

In the present study, the (+) and (–) isomers of amphetamine and four N-substituted amphetamine derivatives have been used to further characterize substrate properties of importance for the interaction with cytochrome *P*-450. The results support the above conclusion that lipid solubility is of minor importance for deciding the affinity by which such interaction takes place and clearly indicate stereoselectivity in cytochrome *P*-450–substrate interaction.

2. Materials and methods

Unstarved, male rabbits (local strain) weighing 1.5–2.0 kg were used. Animals were sacrificed by a blow on the head and bleeding. The liver was immediately removed, placed in ice-cold 0.25 M sucrose, cut into small pieces and homogenized in 4 vol of 0.25 M sucrose. After sedimentation of cell debris, nuclei and mitochondria by centrifugation at 13,300 *g* for 10 min, the microsomal fraction was pelleted by centrifugation at 105,000 *g* for 60 min. The microsomal pellets were washed once with 0.15 M KCl, resedimented by centrifugation at 105,000 *g* for 30 min and suspended in 0.25 M sucrose at a protein concentration of about 10 mg per ml. Protein concentrations were determined by the method of Lowry et al. [9].

The spectral changes caused by addition of various substrates to a suspension of microsomes were recorded in an Aminco–Chance dual wavelength/split beam recording spectrophotometer. Each cuvette contained a microsomal suspension (5 mg microsomal protein/ml) in 50 mM Tris-HCl–15 mM KCl medium, pH 7.5. The substrates were added to the sample cuvette and equivalent amounts of the solvent were added to the reference cuvette. K_s and ΔA_{max} values were determined according to Schenkman [2].

The lipid solubility of the compounds were determined in a system containing corn oil and 0.5 M potassium phosphate buffer, pH 7.5. The compounds were shaken 1 hr in the oil–buffer system. The concentration of the compounds in the water phase was determined by comparison of the absorption peaks in the 250–260 nm region.

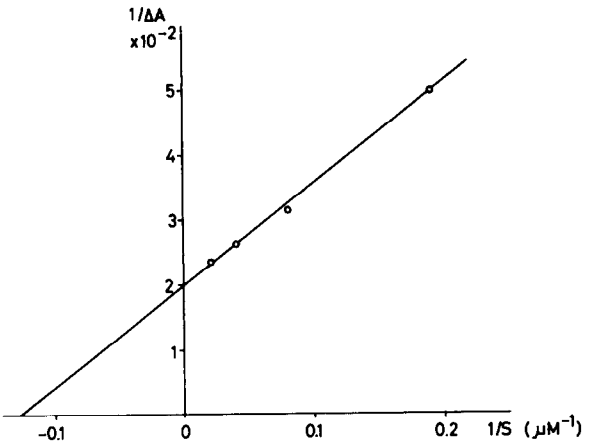

Fig. 2. Determination of the spectral dissociation constant (K_S) of (–) amphetamine. Double reciprocal plots of the absorbance changes at 420 nm and 490 nm recorded upon addition of increasing concentrations of (–) amphetamine to a suspension of rabbit liver microsomes.

3. Results

Axelrod early showed that liver microsomes catalyze the conversion of amphetamine to phenylacetone in the presence of NADPH and molecular oxygen [10]. Gillette et al. [11] subsequently reported that the metabolism of amphetamine is inhibited by carbon monoxide, indicating the involvement of cytochrome *P*-450 in the reaction. Further evidence for such involvement was obtained in the present study.

As shown in fig. 1, the addition of (+) amphetamine at a low concentration to a suspension of rabbit liver microsomes, gave rise to a type I spectral change. When the amphetamine concentration was increased, however, this gradually turned into a type II spectral change, indicative of ferrihemochrome formation. It thus seems obvious that both (+) and (–) amphetamine can interact with cytochrome *P*-450 at two different sites; a high-affinity site to produce the type I spectral change (K_S values of 12 μM and 8 μM, respectively (fig. 2 and table 1)), and a low-affinity site to produce the type II spectral change (K_S values of approx. 4.5 mM and 0.5 mM, respectively (not documented)). As expected, we found amphetamine metabolism to be optimal at concentrations giving rise to the type I spectral change. A comparison of the apparent K_S values for (+) and (–) amphetamine and the apparent

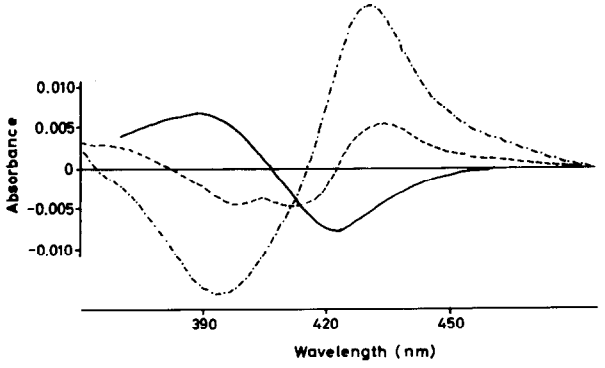

Fig. 1. Difference spectra upon addition of (+) amphetamine to rabbit liver microsomes. (——) 117 μM; (– – – –) 1.5 mM; (–·–·) 7.5 mM (+) amphetamine.

Table 1
Dissociation constants for the cytochrome *P*-450–substrate complex

	(+)	(–)
	K_S (μM)	K_S (μM)
Amphetamine	12	8.0
Methylamphetamine	7.2	6.4
Ethylamphetamine	0.080	9.1
Isopropylamphetamine	0.53	7.2
Dimethylamphetamine	10	8.3

Table 2
Lipid solubility of amphetamine and some amphetamine derivatives.

	% in oil phase	
	(+)	(–)
Amphetamine	6	6
Methylamphetamine	5	5
Ethylamphetamine	11	11
Isopropylamphetamine	19	20
Dimethylamphetamine	28	28

Corn oil–0.5 M phosphate buffer pH 7.4.

Michaelis constants, estimated from the formation of phenylacetone, gave the following results: (+) amphetamine, K_s = 12 μM and K_m = 7 μM and (–) amphetamine, K_s = 8 μM and K_m = 6 μM.

Both isomers of the N-substituted amphetamine derivatives gave rise to the type I spectral change in all concentrations investigated. Furthermore, in spite of considerable differences in lipid solubility (table 2) they appeared to interact with similar amounts of the cytochrome *P*-450 present, as judged from the magnitude of the type I spectral change (table 3). The (–) isomers exhibited similar affinities for this interaction, whereas the (+) isomers displayed considerable differences in affinity (table 1), which were, however, not strictly correlated to differences in lipid solubility (cf. tables 1 and 2). Strong evidence for stereoselectivity in cytochrome *P*-450–substrate interaction was obtained with ethyl- and isopropylamphetamine, whose two isomers displayed marked differences in apparent K_s values (table 1).

Table 3
Spectral interactions of some amphetamine derivatives with rabbit liver microsomes.

	(+)	(–)
	ΔA / nmole *P*-450	ΔA / nmole *P*-450
Amphetamine	–	–
Methylamphetamine	0.028	0.026
Ethylamphetamine	0.023	0.028
Isopropylamphetamine	0.026	0.028
Dimethylamphetamine	0.023	0.028

4. Discussion

This study has provided further evidence that basic amines, which can serve as substrates for cytochrome *P*-450, can interact with the hemoprotein to produce the type I spectral change. Such interaction is usually observed at low drug concentrations which, however, seem to be optimal for metabolism. As previously observed with didemethyl-imipramine [12], we found the type I spectral interaction to be overshadowed by a type II spectral change upon increase in drug concentration. A type II spectral change has previously been observed by Fouts et al. upon addition of amphetamine at a high concentration to liver microsomes [13]. These findings are in accordance with the hypothesis that "type I interaction" is of importance for metabolism [4].

Since the magnitude of the type I spectral change obtained at saturating substrate concentration is proportional to the amount of cytochrome *P*-450 present [1], it seems that the N-substituted amphetamine derivatives investigated can react with equal amounts of cytochrome *P*-450, in spite of considerable differences in lipid solubility. This is in contrast to recent findings with a series of barbiturates which, at saturating concentrations, produced different magnitudes of the type I spectral change which showed some correlation with the lipid solubility of the compounds [8]. Whether also amphetamine can interact with the same concentration of cytochrome *P*-450 as the N-substituted derivatives, could not be decided because of the change in the type of spectral interaction that occurred upon increase in concentration of this drug.

No apparent differences in binding affinity were found with the (–) isomers of the investigated amphetamine derivatives in spite of considerable differences in lipid solubility. This observation supports the previous conclusion that other properties besides lipid solubility govern the affinity by which substrates interact with cytochrome *P*-450 [8]. This point is further strengthened by the considerable differences in K_s values obtained with the (+) and (–) isomers of ethyl- and isopropylamphetamine, which, in agreement with a previous observation [14], clearly indicates stereoselectivity in cytochrome *P*-450–substrate interaction.

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