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SHORT COMMUNICATIONS

Metabolism of amphetamine and β,β-difluoroamphetamine in phenobarbital-treated rats

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WE HAVE previously reported that difluoro substitution on the β carbon of amphetamine alters both tissue distribution and metabolism in rats.[1,2] The alterations are probably due to the marked reduction in pK_a that results because the highly electronegative fluorine atoms pull electrons away from the amine nitrogen and make it less able to accept a proton. β,β-Difluoroamphetamine (pK_a 7·0) exists at physiologic pH mainly as a neutral molecule, whereas amphetamine (pK_a 9·5) is almost completely cationic. The rate of metabolism of the two compounds in rats is similar, but the pathway of metabolism is different, being through oxidative deamination for the difluoro compound as contrasted to *para*-hydroxylation for amphetamine. In this communication, we are reporting that the rate of deamination of difluoroamphetamine (and amphetamine) *in vitro* by rat liver microsomes is increased after phenobarbital treatment and that the half-life of β,β-difluoroamphetamine (but not amphetamine) in tissues is strikingly reduced in phenobarbital-treated rats.

For the studies *in vitro*, liver microsomes were prepared at 0° by standard sedimentation procedures in 0·25 M sucrose. The washed microsomal fractions were resuspended in 1·15% KCl. Incubation mixtures (5 ml total) contained 10 μmoles $MgCl_2$. 2 m-moles phosphate buffer, pH 7·4, 2 μmoles NADPH, 20 μmoles glucose 6-phosphate, 8 units of glucose 6-phosphate dehydrogenase, 5 μmoles of the amine substrate, and 1 ml of 1·15% KCl containing microsomes equivalent to 1 g liver. The reection mixture was incubated with shaking in air at 37° for 30 min. Previous work has shown that the metabolites isolated *in vitro* are phenylacetone and phenylacetone oxime from amphetamine, and β,β-difluorophenylisopropanol and β,β-difluorophenylacetone oxime from β,β-difluoroamphetamine.[3]

For the phenylacetone and β,β-difluorophenylisopropanol determinations, reactions were terminated by the addition of 1 ml of 1·0 N HCl. The reaction mixtures were extracted twice with 4 ml *n*-butyl chloride and the combined extracts were evaporated to dryness. The residue was dissolved in 100–200 μl *n*-butyl chloride and 1·2 μl was injected into a Hewlett Packard 7610A gas chromatograph containing a 4-ft U-shaped column packed with 3% UC-W-98 on diataport S. The temperatures used were: flash heater, 110°; column, 85°; and flame detector, 130°.

Oxime concentrations were determined following *n*-butyl chloride extractions at neutral pH. After evaporating to dryness, 100–200 μl Regisil was added to the extracts in order to convert the oximes to their corresponding trimethylsilyl derivatives. To insure complete derivatization, the mixtures were allowed to stand for 20 min at room temperature.

Using the above gas–liquid chromatography (GLC) conditions, the retention times of the amphetamine metabolites (phenylacetone and phenylacetone oxime) were 1·1 and 6·4 min respectively. The retention times of the β,β-difluoroamphetamine metabolites were β,β-difluorophenylisopropanol 1·3 min and oxime 3·8 min. In order to quantitate the amount of product formed, the GLC peak heights of the metabolites were compared to the peak heights obtained with known amounts of phenylacetone and phenylacetone oxime. There were no interfering peaks when microsomes from normal or induced rats were incubated without substrate.

For studies *in vivo*, male Wistar-derived albino rats weighing approximately 150 g were used. *dl*-Amphetamine sulfate (Chemicals Procurement Laboratories) or *dl*-β,β-difluoroamphetamine hydrochloride was injected at 0·1 m-mole/kg at 8 a.m., and groups of five rats were killed at 60, 100, 140 and 180 min thereafter. Drug levels in brain, liver and epididymal fat were measured spectrophotometrically with the methyl orange method of Axelrod,[4] as modified by Dubnick *et al.*[5] Sodium phenobarbital was injected i.p. at 40 mg/kg twice daily (8 a.m. and 4 p.m.) for 4 days preceding the day of the amine injection or microsome preparation.

Table 1 shows the rate of formation *in vitro* of deaminated metabolites from amphetamine and β,β-difluoroamphetamine. With microsomes from untreated rats, the metabolism of β,β-difluoroamphetamine was faster than that of amphetamine. Phenobarbital pretreatment increased the rate of metabolism of both amines, though amphetamine deamination even after phenobarbital treatment was still slow.

TABLE 1. COMPARATIVE RATES OF OXIDATIVE DEAMINATION OF AMPHETAMINE AND β,β-DIFLUOROAMPHETAMINE BY RAT LIVER MICROSOMES FROM CONTROL AND PHENOBARBITAL-TREATED RATS*

Substrate	Group	Product formed (nmoles/g liver/30 min)		
		Ketone (alcohol)	Oxime	Total
Amphetamine	Control	7·2 ± 0·95	5·6 ± 0·20	12.8
	Phenobarbital	20·2 ± 1·34	24·8 ± 11·7	45·0
Difluoroamphetamine	Control	8·2 ± 1·03	63·8 ± 5·12	72·0
	Phenobarbital	90·5 ± 12·20	316·2 ± 44·7	406·7

* Mean values ± standard errors for five rats per group are shown.

Groppetti and Costa[6] had earlier shown that treatment of rats with phenobarbital or with other inducers of hepatic microsomal enzymes did not detectably influence the rate of metabolism of amphetamine. Amphetamine is metabolized primarily by *para*-hydroxylation in the rat,[4] in contrast to other species. The results of Groppetti and Costa[6] imply that (a) the major metabolic route, *para*-hydroxylation, is by a non-inducible process, and (b) inducible pathways such as deamination play an insignificant role in amphetamine metabolism in rats so that an alteration in their rate is undetected in overall biologic half-life. On the other hand, since β,β-difluoroamphetamine is metabolized primarily by deamination, then the induction of microsomal deamination ought to shorten the biologic half-life of that drug.

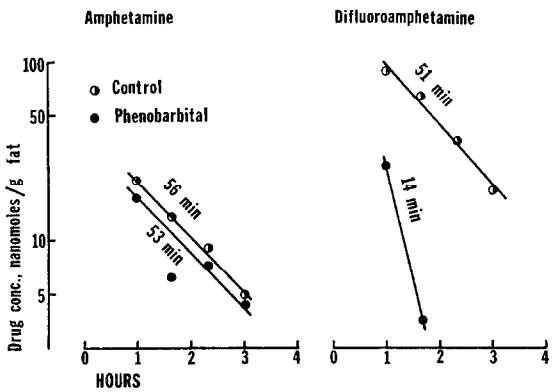

FIG. 1. Drug levels in epididymal fat. Each point represents the mean value for five rats. Half-life values are shown with each line. Experimental details are given in the text.

Figure 1 shows that indeed the levels of β,β-difluoroamphetamine in fat and the rate of decrease of those levels during the time period studied were changed by phenobarbital pretreatment. The calculated half-life for β,β-difluoroamphetamine in fat of control animals was 51 min, about the same as for amphetamine. In phenobarbital-treated rats, the half-life of β,β-difluoroamphetamine was sharply reduced (to about 14 min), whereas the half-life for amphetamine was not appreciably changed (53 min). The tendency for β,β-difluoroamphetamine to localize in fat to a greater degree than does amphetamine is apparent from the data for control animals in Fig. 1; levels of the difluoro compound were much higher than were those of amphetamine.

Drug levels in liver, presumably the major site of metabolism of the drugs, are shown in Fig. 2. Again, the levels of β,β-difluoroamphetamine were lower in phenobarbital-pretreated rats, and the disappearance of the drug was much faster than in controls. Amphetamine levels and rate of disappearance were nearly identical in the two groups. In liver, β,β-difluoroamphetamine levels were slightly lower than those of amphetamine, in agreement with earlier results on the tissue distribution of the two drugs.[1,2]

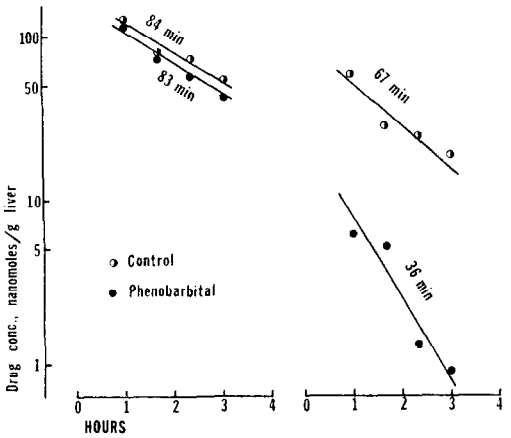

FIG. 2. Drug levels in liver. Conditions as in Fig. 1.

We also measured drug levels in brain in the experiment from which the data for Figs. 1 and 2 were derived. Amphetamine levels were not different in the two groups (half-life 46 min in controls and 52 min in phenobarbital-treated rats). β,β-Difluoroamphetamine levels were low even in the controls—because the drug distributes more to other tissues[1,2]—so that an accurate half-life could not be calculated; in the phenobarbital-treated rats, levels of β,β-difluoroamphetamine were not detectable, once more indicating the more rapid removal of the drug from tissues of phenobarbital-treated rats.

Our results show a marked disparity in the effect of phenobarbital pretreatment on the metabolism of amphetamine and β,β-difluoroamphetamine. The disparity seems to be fully understandable on the basis of different routes of metabolism of the two drugs in rats. The present findings support earlier evidence that the major metabolic route for β,β-difluoroamphetamine in rats is by a different pathway (deamination) than that for amphetamine (*para*-hydroxylation). The findings indicate that the deamination pathway is increased by phenobarbital pretreatment and that the *para*-hydroxylation pathway is unaffected. The latter finding agrees with the results obtained earlier by Groppetti and Costa,[6] who called attention to the peculiar properties of the amphetamine-hydroxylating system. So far as we are aware, no one has yet demonstrated the hydroxylation of amphetamine to be microsomal or even to occur *in vitro* with liver homogenates. Possibly the enzyme responsible is not in hepatic microsomes. On the other hand, the metabolism of β,β-difluoroamphetamine appears to be typically a hepatic microsomal process, inducible by phenobarbital and inhibited by β-diethylaminoethyl diphenylpropyl acetate (SKF-525A).[2]

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