J. Pharm. Pharmac., 1968, **20**, 107–110 Received July 21, 1967

The hydrolysis of acetylsalicylic acid by liver microsomes

J. F. HOWES AND W. H. HUNTER

Pretreatment of rats and guinea-pigs with phenobarbitone or phenylbutazone leads to a decrease in the rate of hydrolysis of acetylsalicylic acid *in vitro* by liver microsomes, treatment with phenacetin does not.

HYDROLYSIS of acetylsalicylic acid *in vivo* is known to occur in serum (Augustinsson, 1948) and in liver and kidney (Ecobichon & Kalow, 1962). A comparative study of hydrolysis rates of acetylsalicylic acid in serum from rats, rabbits, guinea-pigs and man was reported by Morgan & Truitt (1965). The effect of treatment with other drugs upon the hydrolysis of acetylsalicylate has not been reported but Burns, Cucinell & others (1965) found that blood levels of aspirin did not alter as a result of its continued administration. We report the hydrolysis of acetylsalicylic acid in liver microsomes from rats and guinea-pigs pretreated with phenobarbitone, phenylbutazone or phenacetin.

Experimental

MATERIALS AND METHODS

Phenobarbitone sodium was administered intraperitoneally, as an aqueous solution, at 50 mg/kg to male Wistar rats and to guinea-pigs. Phenacetin and phenylbutazone at the same dose level were administered as suspensions in 0·2M Tris buffer (pH 7·6). Suspensions were prepared by ultrasonic disintegration of the solids in buffer solution. The animals were killed at suitable intervals after the administration of a single dose of drug or after the appropriate dose in the experiments involving multiple doses. Blood samples were obtained by severing the jugular vein immediately after killing the animals. The livers were removed, weighed and homogenized in a Waring-Blender (15 sec at low speed) with two volumes of isotonic potassium chloride solution. The homogenate was centrifuged at 9,000 *g* for 30 min to remove nuclei, mitochondria and other cell debris. The supernatant was centrifuged 1 hr at 140,000 *g* at 0–4°. The clear, soluble fraction was decanted from the microsomal pellet and this pellet resuspended in a volume of 0·2M Tris buffer equal to the volume of the discarded layer.

A 1·0 ml sample of the microsome suspension was diluted to 100 ml with distilled water and the protein content determined colorimetrically (Lowry, Rosebrough & others, 1951). Fifty ml of a solution of sodium carbonate 2% w/v and sodium tartrate (0·02% w/v) in 0·1N sodium hydroxide solution were added to 1 ml of copper sulphate solution (0·5% w/v $CuSO_4,5H_2O$) and 5·0 ml of this mixed solution were mixed with 1·0 ml

From the Department of Pharmacy, Chelsea College of Science and Technology (University of London), Manresa Road, London, S.W.3, England.

of the microsome suspension. After 10 min, 0·5 ml of Folin and Ciocalteau reagent (5 ml BDH stock solution diluted to 12·0 ml) was added and the solutions allowed to stand 1 hr. The absorbance of the solutions was measured at 750 mμ. Solutions of bovine serum albumin (BDH) containing 100 μg/ml and 200 μg/ml were used, at the same time, to calibrate the absorbance readings.

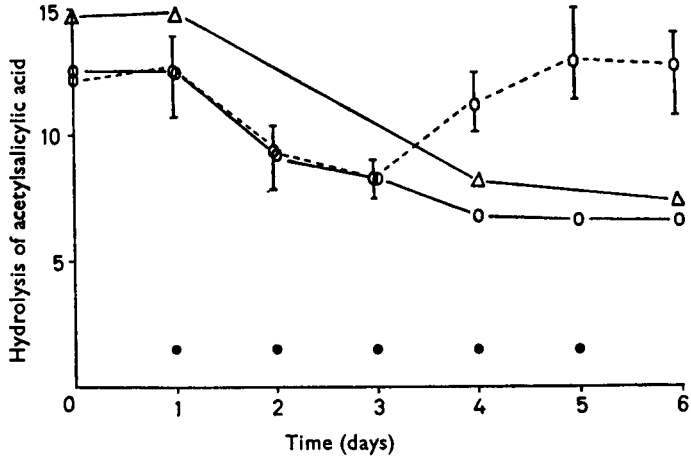

FIG. 1. Solid lines show the effect of phenobarbitone administration (days 1–5) on the hydrolysis of acetylsalicylic acid in microsomes of rat (O) and guinea-pig (Δ) liver. Broken line shows the effect of a single dose of 50 mg/kg on day 1 of phenobarbitone on the hydrolysis of acetylsalicylic acid in rat liver microsomes. Hydrolysis expressed as μg salicylate formed/mg protein/min. Each point is the mean of six experiments.

The microsomal suspensions were diluted with Tris buffer (0·2M) to contain 1 mg of protein per ml. These solutions were then allowed to stand for 30 min to allow the enzymes to re-associate after dilution. It was found that freshly diluted suspensions of microsomes had much lower activity in hydrolysing acetylsalicylate. The acetylsalicylic acid esterase activity of these preparations was then determined in the following way.

Cuvettes were prepared as follows: (1) containing 3·0 ml of 0·2M Tris buffer and used to set the base-line absorbance; (2) containing 0·1 ml of the microsomal suspension (1 mg protein/ml), 1·0 ml of 0·2M Tris buffer, 0·9 ml distilled water and 1·0 ml of acetylsalicylic acid solution (10^{-3}M) (this cuvette measured the rate of enzymic hydrolysis); (3) containing the same reagents as (2) but without the microsomal suspension, which was replaced by 0·1 ml of distilled water. This cuvette measured the slow spontaneous hydrolysis of acetylsalicylic acid in the buffer solution.

The absorbance of all three cuvettes at 300 mμ was determined in a Gilford-Unicam dual wavelength spectrophotometer at 37° over 30 min and the extent of enzymic hydrolysis measured by the difference between the absorbance of cuvette (2) and cuvette (3).

The pH of all solutions was checked before and after the incubation. The hydrolysis of acetylsalicylic acid in serum from treated and untreated animals was measured by the method of Morgan & Truitt (1965). Phenobarbitone, phenylbutazone or phenacetin at levels up to 5 μg/ml had no effect *in vitro* upon the rates of hydrolysis of acetylsalicylic acid by either microsomes or by serum.

Results

The effect of phenobarbitone treatment upon the hydrolysis of acetylsalicylic acid by microsomes from rat and guinea-pig liver is shown in Fig. 1. Fig. 1 also shows the effect of a single dose of phenobarbitone, in

TABLE 1. THE RATES OF HYDROLYSIS OF ACETYLSALICYLIC ACID BY RAT LIVER MICROSOMES, 48 HR AFTER INTRAPERITONEAL ADMINISTRATION OF A SINGLE DOSE OF VARIOUS DRUGS

Drug	Dose mg/kg	Rate of hydrolysis*
Control	—	12·4
Phenobarbitone	50	8·2
Phenylbutazone	,,	6·7
Phenacetin	,,	12·6

* Expressed as μg salicylate formed/mg protein/min.

rats, upon microsomal hydrolysis of acetylsalicylic acid and illustrates the recovery to normal levels after 3–4 days. Table 1 shows the effects of pretreatment with various drugs on the rate of hydrolysis of acetylsalicylate by microsomes of rat liver.

Discussion

The hydrolysis of acetylsalicylic acid in various tissues of experimental animals and of man has been extensively studied (for references see Morgan & Truitt, 1965) but the effect of drug treatment on this hydrolysis has not been reported. The administration of compounds that are oxidized in the liver is known to cause marked changes in the level of microsomal oxidizing enzymes but the effect upon other microsomal enzyme activities has not been so thoroughly studied. Remmer (1964) reported a slight increase in the hydrolysis of procaine by microsomes from dogs pretreated with phenobarbitone and a slight decrease in the case of rats similarly pretreated. Orrenius & Ericsson (1966) reported decreased levels of glucose 6-phosphatase, ATP-ase and NADH cytochrome C reductase; this decrease could be adequately explained by increased non-enzymic protein levels in the liver microsomes of treated animals.

The present work shows that even a single dose of phenobarbitone or phenylbutazone decreased the ability of liver microsomes to hydrolyse acetylsalicylic acid (Fig. 1). The activity is restored to normal levels in 3–4 days. On continued treatment with phenobarbitone a decreased level of hydrolysing activity is maintained so long as the administration of phenobarbitone is continued (Fig. 1). This decrease of 40–45% in

the capacity of the microsomes to hydrolyse acetylsalicylate is greater than that reported for any other hydrolytic enzyme activity in microsomes. Such a decrease could not be adequately explained by the synthesis of non-enzymic protein in the liver. The administration of phenobarbitone at 100 mg/kg produces a 50% increase in microsomal protein in the rat after five daily doses (Orrenius & Ernster, 1964). A single dose of 90 mg/kg gave a 5% increase in microsomal protein (Kato, 1963). The acetylsalicylic acid esterase activity of serum from treated and untreated rats was identical and the hydrolysis in serum therefore was not affected by the administration of phenobarbitone.

Our results indicate that the hydrolysis of acetylsalicylic acid in isolated liver microsomes is suppressed when phenobarbitone or phenylbutazone is administered but not when phenacetin is used. Both phenobarbitone and phenylbutazone produce significant increases in the levels of components of the oxidative metabolic system in liver microsomes whereas phenacetin does not (Conney, Davidson & others, 1960). The decrease in hydrolysis of acetylsalicylate appears therefore to be associated with gross increases in the drug oxidative activity.

The administration of so-called "inducing" drugs in combination with acetylsalicylic acid might therefore be expected to affect the rate of hydrolysis of acetylsalicylic acid in the liver. The effect is of particular interest since drugs such as phenylbutazone are often used in conjunction with acetylsalicylic acid treatment.

References

Augustinsson, K. B. (1948). *Acta physiol. scand.*, **15**, *Suppl.* 52, 182–188.
Burns, J. J., Cucinell, S. A., Koster, R. & Conney, A. H. (1965). *Ann. N.Y. Acad. Sci.*, **123**, 273–286.
Conney, A. H., Davidson, C., Gastel, R. & Barns, J. J. (1960). *J. Pharmac. exp. Ther.*, **130**, 1–7.
Ecobichon, D. J. & Kalow, W. (1962). *Biochem. Pharmac.*, **11**, 573–578.
Kato, R. (1963). *Experientia*, **19**, 30–33.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.*, **193**, 265–273.
Morgan, A. M. & Truitt, E. B. (1965). *J. pharm. Sci.*, **54**, 1640–1646.
Orrenius, S. & Ericsson, J. L. E. (1966). *Biochem. biophys. Res. Commun.*, **16**, 60–65.
Orrenius, S. & Ernster, L. (1964). *J. cell. Biol.*, **31**, 243–256.
Remmer, H. (1964). *Proc. Europ. Soc. for Study of Drug Toxicity*, IV, 57–77.