Journal of Medical Genetics (1976). **13,** 17–19.

Polymorphic acetylation of nitrazepam

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Summary. Nitrazepam is metabolized in part by nitro-reduction to an amine followed by acetylation. This acetylation step has been shown to be under the control of the same genetic polymorphism as sulphamethazine (syn: sulphadimidine).

A genetic polymorphism has been shown to exist for the metabolism of isoniazid (Knight *et al*, 1959; Price Evans *et al*, 1960). The polymorphism has been shown to control the enzyme N-acetyltransferase located in human liver and intestinal mucosa (Price Evans and White, 1964; Jenne, 1965). The acetylation of sulphamethazine (syn: sulphadimidine, sulfapyridine, hydralazine, dapsone, and procainamide) has also been shown to be controlled by the same genetic polymorphism (Price Evans and White, 1964; Schröder and Price Evans, 1972; Karlsson and Molin, 1974; Gelber *et al*, 1971). There is suggestive clinical evidence that phenelzine may also be subject to the same polymorphism (Price Evans *et al*, 1965).

The hypnotic nitrazepam (1,3-dihydro-7-nitro-5-phenyl-2H-1,4-benzodiazepin-2-one) has been shown to be metabolized in man in part by the successive enzymic biotransformation steps of nitro-reduction to an amine followed by acetylation to the acetamido compound (Beyer and Sadee, 1969; see Fig. 1). The purpose of the present work was to find if the acetylation of reduced nitrazepam in man is under the control of the genetic polymorphism.

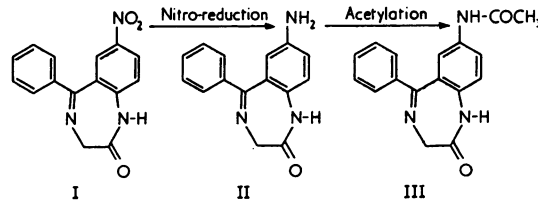

FIG. 1. A part of the scheme of metabolic biotransformation of nitrazepam in man. I—nitrazepam; II—amino metabolite; III—acetamido metabolite.

Methods

Experimental subjects. Volunteers who gave fully informed consent were healthy medical students, technicians, and doctors. They all participated in two test procedures on two occasions at least two weeks apart. The two procedures were first, a standard sulphamethazine phenotyping test (Price Evans, 1969) and secondly, a test in which a standard single oral dose of nitrazepam was followed by a single urinary collection.

Acetylator phenotyping. Acetylator phenotyping was carried out using sulphamethazine (syn: sulphadimidine) according to the procedure of Price Evans (1969). Serum samples were labelled 'sulphamethazine serum' and a random code number, and urine samples were labelled 'sulphamethazine urine' and a random code number, so that analyses were performed blind.

Nitrazepam test. Volunteer subjects were instructed to fast for at least 3 h following the last evening meal. They then swallowed 10 mg nitrazepam (Mogadon, Roche) as crushed tablets with a cupful of water before retiring. They were instructed to remain fasting until breakfast-time. They were allowed their usual breakfast, their liquid intake being limited to 2 cups of tea or coffee. A urine sample was collected from 8 to 11 h after nitrazepam ingestion. The specimen was brought immediately to the laboratory without preservative, and was either analysed the same day or stored at $-20°$ C to await analysis. These urine specimens were labelled 'nitrazepam urine' and random code numbers were assigned quite different from those used for sulphamethazine specimens, so that the analyst did not know to which sulphamethazine serum or urine specimens they were related.

Analysis of nitrazepam urines. When the urine sample had been stored at $-20°$ C it was thawed at $+40°$ C and then kept at this temperature for 15 min with repeated shaking.

The analysis was a modification of that of Rieder (1965).

To 20 ml urine 400 mg MgO was added and shaken for

Received 6 January 1975.

30 s to raise the *p*H to 10. The metabolites were extracted in 40 ml dichloromethane and ethyl acetate extraction mixture (2:1 by vol) by shaking for 10 minutes. The organic phase was removed as completely as possible, concentrated to dryness in a rotary evaporator, and then redissolved in 9.5 ml of the same extraction mixture with 5 min thorough shaking. A 10 min shaking with 10 ml 5% aqueous borax solution (saturated beforehand with the extraction mixture as above) then followed. 8 ml organic phase was transferred to a tube and 7 ml 0.2 nmol/l HCl was added and mixed by shaking for 10 min. 3 ml acid extract was transferred into each of two tubes—one for the determination of the amino metabolite ('free') and the other for the amino plus acetamido metabolites ('total').

For the determination of the amino metabolite 0.2 ml 0.4% aqueous sodium nitrite (freshly made up) was added and after mixing left to stand at 0° C for 5 min. Then at room temperature 0.2 ml 2% sulphamic acid was added, mixed, and left standing at room temperature for 2 min with repeated shaking in order to facilitate escape of excess of NO_2 gas. Finally 0.2 ml 0.4% α-naphthylethylenediamine dihydrochloride was added, mixed, and left standing for at least 20 min. The extinction was measured at 555 nm.

For the determination of total amino metabolite (ie amino plus acetamido) the tube containing the 3 ml acid extract was sealed tightly with aluminium foil and then hydrolysed in a boiling water bath for 50 min. The tubes were cooled in water at room temperature and then analysis proceeded as for the free amino metabolite.

Unknown urine samples (*ab initio*), reagent blanks (in distilled water), and a blank urine (always from AKMBK who had not taken nitrazepam) were all processed in duplicate in each analytical run.

Standard solutions were composed of 19.9 ml blank urine to which was added 0.1 ml solutions of 100, 200, 300, and 400 μg/ml concentrations of amino metabolite in 50% ethanol giving final concentrations of 0.5, 1.0, 1.5, and 2.0 μg/ml in blank urine. An array of standards made up in the same way was used for determination of both 'free amino' and 'total' (ie, amino plus acetamido) metabolites. All standards were put up in duplicate in each analytical run.

The concentrations of free amino metabolites (F) and total amino metabolites (T; ie, amino plus acetamido) in unknown urine samples were calculated from the regression of extinction (y) on concentration (x) obtained in the same analytical run. There was very little variability in these regression lines between analytical runs. Percentage acetylation was computed as T−F/T%.

Results

The sulphamethazine phenotyping procedure differentiated clearly between the rapid and slow acetylator phenotypes (Fig. 2).

With regard to the nitrazepam results, Fig. 3 demonstrates that the percentage acetylation of the amino metabolite of nitrazepam in urine corresponded to the acetylator phenotype. In this sample of healthy volunteer subjects, there was no overlap for this value between the two acetylator phenotypes.

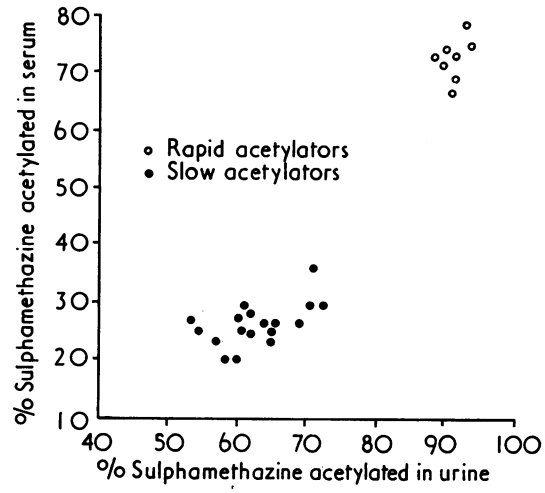

FIG. 2. The results of the sulphamethazine phenotyping procedure in the population of healthy volunteer subjects studied.

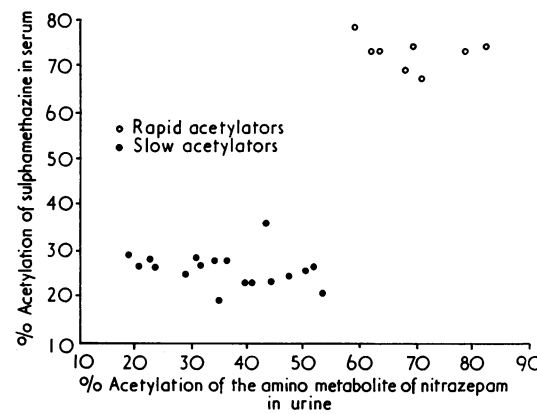

FIG. 3. The correlation of percentage acetylation of the amino metabolite of nitrazepam in urine, with the phenotyping result (as shown in Fig. 2) of percentage acetylation of sulphamethazine in serum on the same subjects on a different occasion.

It is clear, therefore, that the acetylation step in nitrazepam metabolism (Fig. 1) is under the control of the acetylation genetic polymorphism.

Discussion

Nitrazepam is a widely used medication. An estimated 3 122 000 prescriptions were written for this drug by family doctors in 1970 in the UK (Committee on Safety of Medicines, 1974).

It has been estimated in one healthy adult man only (whose acetylator phenotype was unknown)

that after a single 10 mg oral dose about 2% of the dose was excreted in the urine as the amino compound and about 5% as the acetamido compound (Rieder and Wendt, 1971).

The psychological effects of the amine metabolite appear to be unknown.

It is possible that the genetic acetylator polymorphism may influence the hypnotic therapeutic effects, and also the adverse effects of nitrazepam medication in man.

We would like to thank: Miss M. F. Bullen SRN, Department of Medicine, University of Liverpool, for conducting the tests on the volunteers; Dr Peter Nowell of the Department of Pharmacology and Therapeutics, University of Liverpool, for helpful suggestions regarding the analytical techniques for nitrazepam metabolites, and Dr R. F. Long, Hoffman-La Roche Ltd, for information and samples of pure nitrazepam and the amino and acetamido metabolites.

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