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THE METABOLISM OF SOME 4-ACETYL-1-NAPHTHYL ETHERS IN THE RAT AND THEIR EFFECT UPON LIVER MICROSOMAL OXIDATION

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Abstract—The excretion of two ethers of 4-acetyl-1-naphthol and of their metabolites has been examined in rats. The results are used to explain the relative capacities of the compounds to stimulate oxidative metabolism in liver microsomes from treated animals.

IN A PREVIOUS paper[1] we reported the stimulation of strychnine metabolism by a series of anti-convulsant compounds derived from 4-acetyl-1-naphthol. The pharmacological properties of one compound in this series, 1-(4-acetyl-1-naphthoxy)2-propanol (NPOH) have been studied in some detail.[2] The metabolic fate of this compound and of the corresponding ketone (4-acetyl-1-naphthoxy), propanone (NPO), was of considerable interest since the two compounds were easily converted one into the other in the soluble fraction of liver supernatant and, presumably, *in vivo*.[1]

Nevertheless, the propanone, NPO, stimulated the oxidative metabolism of strychnine in liver homogenates from pretreated rats to a much greater degree than did the secondary alcohol NPOH.

We wish to report some further studies with these compounds concerning the rates at which they are oxidatively metabolised *in vivo*, the proportions of each compound excreted in oxidised or conjugated form and the way in which these proportions affect microsomal oxidation.

METHODS

The 4-acetyl-1-naphthyl ethers were prepared as described previously.[2]

Excretion of 4-acetyl-2-naphthyl derivatives

Male Wistar rats, weighing about 300g, were used in all experiments and compounds were administered at a dose of 250 mg/kg as suspensions in 1 ml arachis oil either by i.p. injection or by stomach tube. The rats were transferred to cages and urine and faeces collected at intervals up to 80 hr. 4-Acetyl-1-naphthol and NPOH, when present as metabolites in urine or faeces were always conjugated as the glucuronides and no free phenol or NPOH could be detected in the excreta by TLC of extracts. The volume of each urine sample was therefore made up to 30 ml with distilled water and an aliquot (5 ml) treated with 5·0 ml of concentrated hydrochloric acid at 100° for 1 hr. These solutions were cooled, made up to 20 ml with 25% sodium hydroxide solution and extracted 3 times with 20 ml portions

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of ether. At this stage the ether contained the neutral metabolites and the aqueous phase was an alkaline solution of 4-acetyl-1-naphthol. The presence of the metabolites was confirmed by TLC[1] and both solutions were assayed for their metabolite content as follows:

(1) The ether extract was dried, evaporated to dryness and the residue dissolved in 10·0 ml of spectroscopic ethanol. Blank extracts were made from urine samples of untreated animals. The absorbance of the solutions was measured at 318 mμ in a Unicam SP800 recording spectrophotometer. The concentration of NPOH in the ethanol extract was determined from a standard calibration curve. This method was found to give 101–106 per cent recovery of NPOH from urine.

(2) The aqueous layer was acidified by addition of 5·0 ml of concentrated hydrochloric acid and a 10·0 ml aliquot was shaken with 25 ml of heptane containing 1·5% isoamyl alcohol, for 15 min. Twenty ml of the heptane layer was shaken with 4·0 ml of 0·1 N sodium hydroxide solution and the absorbance of this alkaline aqueous extract measured at 382 mμ. This procedure gave 95–102 per cent recovery of 4-acetyl-1-naphthol when compared with blank determinations on urine from untreated animals. The concentration of 4-acetyl-1-naphthol was calculated from a calibration curve.

Faeces were steeped in 30 ml of a 50% ethanol-water mixture for 24 hr and the mixture made up to 100 ml with distilled water. A 5·0 ml aliquot of this solution was acidified with 5·0 ml of concentrated hydrochloric acid and heated 1 hr at 100°. NPOH and 4-acetyl-1-naphthol were assayed as described above.

Stimulation of microsomal oxidation of strychnine by oral administration of NPO and NPOH

The compounds were administered by stomach tube at 250 mg/kg as suspensions in 1 ml arachis oil. Control animals received only arachis oil and all animals were allowed to feed and drink normally. The animals were killed 48 hr after this single dose, livers were removed and treated as previously described.[1] The metabolism of strychnine by liver supernatant fraction from treated and control rats was determined by the method used previously[1] and results are recorded in Table 3.

Glucuronyl transferase activity in liver microsomes and in intestinal mucosa

Liver microsomes were prepared as described.[1]

Intestinal mucosa were prepared by removing the whole small intestine of rats and washing it through with isotonic potassium chloride solution. The intestine was opened, pinned out on a board and the mucosa scraped free from the muscular layers with a scalpel. The mucosa were then weighed and homogenised with 2 vol. of isotonic potassium chloride solution in an Ultra-Turox homogeniser. Protein was determined in mucosal and microsomal homogenates by the method of Lowry *et al.*[3]

1·0 Ml of tissue preparation (mucosal or microsomal) was incubated in the following medium: magnesium chloride solution (0·1 M) 0·1 ml; uridine diphosphate glucosiduronic acid (UDPGA) (1 mg/ml) 0·5 ml; 2-aminophenol (0·12 mg) 0·5 ml and Sorensens phosphate buffer (0·05 M, pH 7·4) 0·9 ml. The solution was incubated for 2 hr at 37° then 1·0 ml of 7·5% trichloracetic acid was added and the solution centrifuged for 5 min at 5000 *g*. To the supernatant was added 1·0 ml of 0·05% sodium nitrite solution and, 5 min later, 1·0 ml of 0·5% ammonium sulphamate solution. After a further 5 min 1·0 ml of 0·1% naphthalene ethylenediamine solution

was added and the solution incubated 2 hr at 25°. The absorbance was measured at 550 mμ and the concentration of 2-aminophenol glucuronide determined from a calibration curve.

When NPOH was used as substrate it was added as a saturated solution in the buffer. After incubation the solution was treated with trichloracetic acid to precipitate protein and the supernatant adjusted to pH 9. The solution was extracted 3 times with 10 ml of ether and the combined extracts were evaporated and dried at 30°. The residue was taken up in 5·0 ml of spectroscopic ethanol and the absorbance measured at 318 mμ. Blank determinations were conducted without UDPGA and the differences in absorbance were a measure of the glucuronide formed.

RESULTS

Excretion of NPOH after i.p. administration

About 35 per cent of the administered dose was excreted in the faeces and a similar quantity in the urine (Table 1). The time course of excretion in rats is shown in Fig. 1. During the first 24 hr the rats slowly excreted 4-acetyl-1-naphthol as the glucuronide accompanied by a steady excretion of NPOH, also as the glucuronide. After 24 hr the proportionof the conjugated phenol increased although traces of both metabolites were still present at 96 hr. Total recovery of both glucuronides at this time accounted for about 70 per cent of the administered dose.

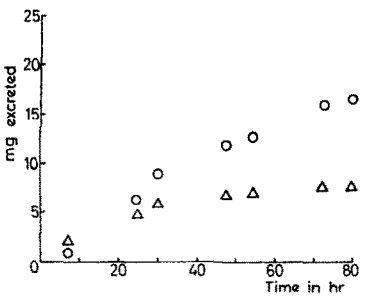

FIG. 1. Excretion of NPOH (△) and 4-acetyl-1-naphthol (○) in urine of rats after i.p. administration of 75 mg of NPOH.

TABLE 1. EXCRETION OF NPOH AND 4-ACETYL-1-NAPHTHOL AS GLUCURONIDES 80 hr AFTER ADMINISTRATION OF NPO OR NPOH (75 mg–300 g RATS)

Route of administration	Compound administered	Glucuronide excreted	Quantities excreted (mg)* urine	faeces	total
i.p.	NPOH	NPOH	7·9 (7·8–8·0)	7·8 (7·5–8·0)	15·7
		4-acetyl-1-naphthol†	20·6 (20·1–22·0)	17·0 (16·3–17·6)	37·5
,,	NPO	NPOH‡	3·8	4·8 (4·6–5·2)	8·6
		4-acetyl-1-naphthol‡	28·6 (28·0–29·1)	19·1 (18·7–19·3)	47·7
oral	NPOH	NPOH	44·0 (43·7–44·1)	7·9 (7·8–8·0)	51·9
		4-acetyl-1-naphthol†	2·8 (2·6–3·2)	0	2·8
,,	NPO	NPOH‡	4·0 (3·8–4·1)	3·8 (3·5–4·2)	7·8
		4-acetyl-1-naphthol‡	29·1 (28·8–29·2)	21·2 (20·1–23·1)	50·2

* Determined after decomposition of the glucuronides.
† Calculated as NPOH.
‡ Calculated as NPO.
Each result represents the average of at least six animals.
Figures in brackets are the range of results.

Excretion of NPO after i.p. administration

This compound was excreted quite rapidly as 4-acetyl-1-naphthol glucuronide which comprised almost all of the 70 per cent of the dose that could be accounted for. A minor metabolite in both urine and faeces was the glucuronide of NPOH and no unchanged NPO could be detected in the excreta by TLC of the extracts. The excretion rate and quantities recovered are shown in Fig. 2 and Table 1.

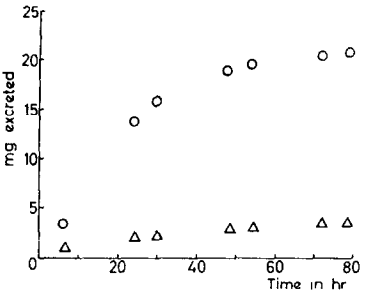

FIG. 2. Excretion of NPOH, (△) and 4-acetyl-1-naphthol (○) in urine of rats after i.p. administration of 75 mg of NPO.

Excretion of NPO and NPOH after oral administration

There was very little qualitative or quantitative difference in the excretion of NPO whether from oral or from i.p. administration. This compound, even an oral administration is absorbed very effectively and is metabolised either by reduction to NPOH, excreted as the glucuronide, or oxidised to 4-acetyl-1-naphthol, also excreted as the glucuronide. This similarity in excretion pattern is evident in the graphs of i.p. administration, Fig. 2, and oral administration, Fig. 4.

When NPOH was administered orally, however, only a small proportion of the drug was oxidatively metabolized (Fig. 3 and Table 1) and most of the material recovered was NPOH glucuronide. Table 2 shows the relative glucuronyl transferase activity of liver microsomes and intestinal mucosa from rats when NPOH was used as substrate. The high proportion of glucuronide formed after oral administration is compatible with the greater activity of glucuronyl transferase in the intestine.

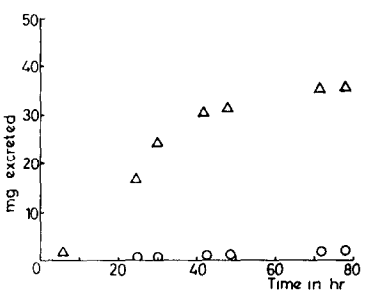

FIG. 3. Excretion of NPOH (△) and 4-acetyl-1-naphthol (○) in urine of rats after oral administration of 75 mg NPOH.

DISCUSSION

The original study on the metabolic fate of these compounds showed that NPO was a much more potent stimulator of oxidative metabolism of strychnine than was

NPOH.[1] Both compounds however were excreted as a mixture of NPOH and 4-acetyl-1-naphthol glucuronides. The much greater stimulation of strychnine oxidation caused by NPO could not be explained by a different pathway for oxidation of this compound. The explanation could however depend upon the ability of the

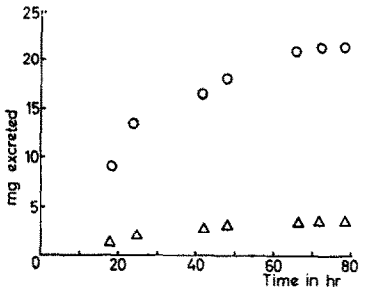

FIG. 4. Excretion of NPOH (△) and 4-acetyl-1-naphthol (○) in urine of rats after oral administration of 75 mg NPO.

TABLE 2. GLUCURONYL TRANSFERASE ACTIVITY OF RAT LIVER MICROSOMES AND INTESTINAL MUCOSA

Substrate	Activity of liver microsomes*	Activity of intestinal mucosa*
2-aminophenol	0.32	4·16
NPOH	0·11	1·72

* Calculated as μg glucuronide formed/mg of protein/2 hr incubation.

compounds *per se* to stimulate microsomal oxidative processes, upon the proportions of the two compounds that were metabolised oxidatively or upon the rate at which they were metabolised which would affect the concentrations available to the liver *in vivo*.

Approximately 60 per cent of an i.p. dose of NPO was accounted for by 4-acetyl-1-naphthol (Table 1) and about 10 per cent by NPOH, excreted as the glucuronides. When NPOH was given i.p., 50 per cent of the dose was recovered as 4-acetyl-1-naphthol glucuronide and 20 per cent as the glucuronide of NPOH. Thus, if stimulation of microsomal oxidation is produced only by compounds that are themselves metabolised by microsomal oxidation then the oxidative metabolism of the extra 10 per cent of the dose in the case of NPO leads to an increase in the degree of stimulation, as measured by strychnine oxidation. This increase we previously showed to be about 100 per cent.[1] The rate of excretion of 4-acetyl-1-naphthol glucuronide is a measure of the availability of each compound to the liver and it is apparent that this rate is much higher for NPO (Fig. 2) than for NPOH (Fig. 1) following i.p. administration. Thus the slight difference (10 per cent) in the proportions of the compounds finally excreted gives but a poor indication of the actual amounts being metabolised oxidatively shortly after administration. For about 30 hr after dosage NPO was being oxidatively metabolised about twice as fast as was

NPOH (Figs. 1 and 2). There is therefore in these two compounds a correlation between a high rate of oxidative metabolism and greater stimulation of the oxidative process.

Availability of a compound to the liver at a sufficient level for a long time is accepted as one necessary criterion for stimulatory activity. The above results may be contrasted with the findings of Remmer[4] who reported that a single dose of hexobarbital was sufficient to stimulate oxidative metabolism of the drug in the dog, a species that metabolises hexobarbital very slowly. In rats and rabbits, which rapidly metabolise hexobarbital, a much longer course of hexobarbital treatment was needed to produce stimulation.

This correlation, in these two compounds, of rapid oxidative metabolism with increased stimulation of oxidative metabolism does not necessarily imply that plasma levels of the compounds are different in the two cases. Indeed the overall amounts of the compounds excreted in urine and faeces are very similar, after i.p. administration of NPO and NPOH; the difference lies in the rate of oxidation rather than in the proportion of the dose oxidatively metabolised.

In order to test the validity of the correlation indicated by i.p. administration, the effect of oral administration of NPO and NPOH was examined. Oral administration of NPO led to a stimulation of strychnine metabolism similar to that produced by i.p. administration. Oral NPOH administration however gave no such increase (Table 3 and ref. 1). This finding is confirmed by the proportions of the compounds that were metabolised by oxidation after oral dosage (Table 1). Only a very small

TABLE 3. RATE OF METABOLISM OF STRYCHNINE BY SUPERNATANT FRACTION OF RAT LIVER (R = 4-ACETYL-1-NAPHTHOL)

Compound administered	μ Mole strychnine metabolised/g liver/hr
None	0·25 (0·23–0·27)
$ROCH_2CH(OH)Me$, NPOH	0·27 (0·25–0·30)
$R.O.CH_2$ CO.Me, NPO	0·70 (0·65–0·72)

Compounds administered at 250 mg/kg orally in arachis oil.
Control animals received only arachis oil.
Each result represents the average of at least six animals.
Figures in brackets represent the range of results.
For full details of method see ref. 1.

proportion of an oral dose of NPOH was excreted as the glucuronide of the oxidation product 4-acetyl-1-naphthol. Excretion of NPOH as the glucuronide after oral administration accounted for most of the dose given (Fig. 3) and may be explained either by glucuronide formation in the liver, or, more plausibly, by the action of glucuronyl transferase in the intestine (Table 2). The excretion pattern of NPO given orally, closely resembled that for i.p. administration (Figs. 2 and 4).

These results indicate that, in these compounds, the route of administration can significantly affect the stimulation of microsomal oxidation and that this stimulation is produced only when the compounds are oxidatively metabolised in the liver. The

question as to whether slow or rapid metabolism of a drug leads to the greatest degree of stimulation of liver microsomal oxidation must await further work with a wider series of compounds.

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