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EFFECT OF ETHER ANAESTHESIA ON PHARMACOKINETICS OF *N*-(2 HYDROXY ETHYL) 2-PHENYL ETHYL CARBAMATE: INHIBITION OF ITS ENTEROHEPATIC CIRCULATION

HOPÉ O. OBIANWU[1]

From Research Laboratories of AB Hässle, Fack, S-431 20 Mölndal 1, Sweden

1 The effect of the ether anaesthesia on the plasma half-life of *N*-(2 hydroxy ethyl) 2-phenyl ethyl carbamate has been studied in rats.

2 The plasma half-life of the carbamate was considerably shorter in animals anaesthetized with ether prior to drug administration than in control rats.

3 The longer plasma half-life of the carbamate in control animals was shown to be due to enterohepatic circulation.

4 Inhibition of this phenomenon by ether was responsible for the shorter plasma half-life of the carbamate in animals anaesthetized with this agent before drug administration.

5 The possible mechanisms by which the ether-induced effect is brought about are discussed.

Introduction

It is now generally recognized that the choice of anaesthetic agent in acute experiments may exert an important influence on the information obtained. Diethylether is commonly used to induce anaesthesia in most laboratory animals in situations where a short anaesthetic effect is desired. Although the animals regain full consciousness within a short time after ether anaesthesia, other important effects may persist for several hours. Full cognizance of this possibility is not often taken.

During the course of combined pharmacokinetic–pharmacodynamic studies in rats of an aromatic carbamate (*N*-(2 hydroxy ethyl) 2-phenyl ethyl carbamate), a potential inhibitor of gastric secretion, it was observed that the plasma half-life of the drug was considerably shorter in experimental animals (operated under ether anaesthesia) than in control animals. Further studies showed that enterohepatic circulation of the drug was responsible for its longer half-life in untreated animals and that ether anaesthesia inhibited this phenomenon. The present studies suggest that ether anaesthesia may have profound effect on the pharmacokinetics of certain drugs.

[1] Present address: Department of Pharmacology, Faculty of Medicine, University of Benin, Benin City, Nigeria.

Methods

Male Sprague-Dawley rats weighing 200-250 g were used except in liver perfusion studies where blood was obtained from animals weighing 300-400 grams.

Ligation of the common bile duct

The animals were anaesthetized with ether (A ether ad Narcosin, Skanska Bomullskrutfabriks AB, Dösjebro, Sweden) and incised about 2 cm paramedially through the right side of the shaved abdominal wall. The loop of the duodenum was pulled through the incision and the common bile duct ligated as near to its entry into the duodenum as possible. The wound was then closed in layers by cotton sutures and the animals were used about 16 h later. Sham operated rats served as controls. The test substance was given via the tail vein.

Ether anaesthesia

Rats were maintained under light ether anaesthesia for 15 min and were given the test substance via the tail vein about 30 min later.

Treatment of rats with antibiotic

Rats were treated with the antibiotic, ampicillin (Doktacillin, Astra, Södertälje, Sweden), 25 mg/rat

in three divided doses on the first day. On the second day each animal was given a single dose of 25 mg at 9 h 00 minutes. The doses were given by stomach tube. The animals were used about 2 h later.

Isolated perfused rat liver

The procedures and apparatus described by Miller, Bly, Watson & Bale (1951) and Bickel & Minder (1970) were adopted with certain modifications. The perfusate consisted of equal volumes of heparinized whole rat blood and Krebs-Henseleit buffer and was circulated by means of a roller pump (MHRE 88, Watson–Marlow Ltd, England). All the tubings were of Silastic (Dow Corning) type.

The PO_2, PCO_2 and pH of the perfusate entering the liver were continuously monitored by means of a flow cuvette (Type DS 66014) containing electrodes, E 5046, E 5036, G 265C and KS 67053 and connected to an Acid-Base analyser (Type PHM 72, Radiometer, Copenhagen). The test drug was added to the perfusate reservoir. Liver was obtained from rats under pentobarbitone anaesthesia.

Bilary excretion

Two rats were anaesthetized with urethane, 1.2 g/kg, intraperitoneally and their bile ducts cannulated; 12 mg/kg of the carbamate dissolved in warm saline was given intravenously via a catheter in the left jugular vein about 30 min after the operation. Bile was collected for a period of 6 hours.

Enzyme hydrolysis of bile

Aliquots (0.2 ml) of bile were mixed with 0.8 ml acetate buffer pH 5 and 50 μl β-glucuronidase/arylsulphatase (Boehringer) and incubated at 37° C for about 4 hours. Control bile samples were treated similarly except that the enzyme was omitted.

Assay of [^{3}H]-N-(2 hydroxy ethyl) 2-phenyl ethyl carbamate

Blood samples were obtained from the heart under light ether anaesthesia. Portions (2 ml) of plasma or perfusate plasma were mixed with 3 ml toluene in glass tubes, saturated with about 3 g sodium chloride and shaken mechanically for about 10 minutes. The tubes were centrifuged and 2 ml aliquots of the toluene phase were mixed with 10 ml of PPO (2,5-diphenyl-oxazole)/POPOP (1,4-di-(2(5-phenyloxazole))-benzene) scintillator (PPO 6 g, POPOP 0.2 g and toluene to 1 litre) in counting vials. Radioactivity was estimated in a Packard Tri-Carb liquid scintillation spectrometer. Quenching was corrected by means of a quench curve. Corrections for extraction losses of 10-20% were made.

The specificity of the method was determined by subjecting the toluene extract of plasma obtained from animals treated with the carbamate, 12 mg/kg, to thin layer chromatography (acetone-benzene; 1 : 1). A single peak of radioactivity with an R_F value of 0.38, identical with that of authentic [^{3}H]-carbamate, was obtained. Tritium labelled *N*-(2 hydroxy ethyl) 2-phenyl ethyl carbamate (labelled at the para position of the benzene ring) was synthesized at our research laboratories and had a specific activity of 625 mCi/mM.

Assay of [^{3}H]-N-(2 hydroxy ethyl) 2-phenyl ethyl carbamate and its metabolites

Aliquots (0.2 g) of samples were oxidized according to the method described by Mahin & Lofberg (1966) except that the samples were mixed with Instagel (Packard) after oxidation. Radioactivity was estimated as above.

Results

Effect of ether anaesthesia on plasma levels of N-(2 hydroxy ethyl) 2-phenyl ethyl carbamate

In rats anaesthetized with ether prior to drug administration, the plasma half-life (β-phase) of the carbamate was about 100 min whereas the levels remained constant or declined very slowly in the control animals (Figure 1). In both the ether-anaesthetized and control animals, the α-phase of the plasma curve lasted up to 2 hours. Thereafter, the plasma levels of both the unchanged drug and total radioactivity continued to decline in ether-treated animals while they remained steady or rose slightly in the control animals (Figure 1). The effect of ether lasted for at least 6 hours. The ratio of unchanged drug to total radioactivity was higher in ether-treated animals than in the control ones, suggesting that this anaesthetic agent might have also inhibited the biotransformation of the carbamate.

Effect of bile duct ligation on the plasma levels of N-(2 hydroxy ethyl) 2-phenyl ethyl carbamate

In an earlier study (Obianwu & Eklund, unpublished observations), it was shown that about 50% of the given dose of the carbamate is

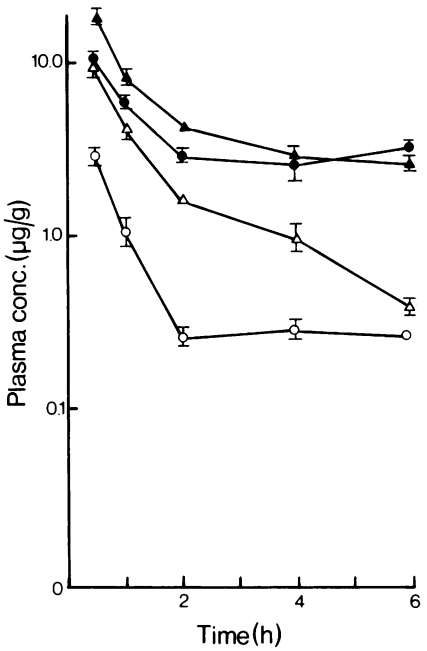

Fig. 1 Effect of ether anaesthesia on plasma levels of [^{3}H]-*N*-(2 hydroxy ethyl) 2-phenyl ethyl carbamate. Rats were maintained under light ether anaesthesia for 15 min and were given the carbamate, 12 mg/kg, via the tail vein about 30 min later. Control animals received only the test substance without ether anaesthesia. Total radioactivity is the activity due to the unchanged drug and its metabolites. Each point is a mean (±s.e.) of four determinations except those at 0.5 and 1 h which were means of seven determinations. The means of the total radioactivity of ether-treated animals (▲) differed significantly from those of the control (●) at 0.5, 1 and 2 h ($P < 0.001$; $P < 0.005$ and $P < 0.001$ respectively) but not at 4 and 6 h ($P > 0.60$ and $P > 0.30$ respectively). The means of the unchanged drug of ether-treated animals (△) differed significantly from those of the control (○) at 0.5, 1, 2, 4 and 6 h ($P < 0.001$; $P < 0.001$; $P < 0.001$; $P < 0.02$ respectively).

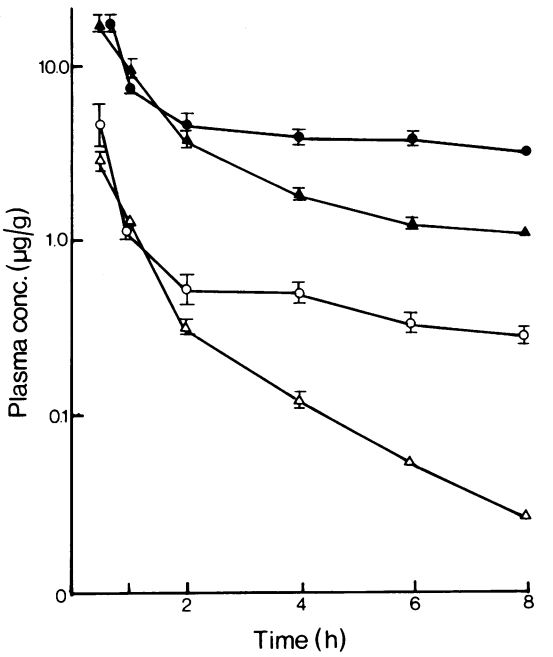

Fig. 2 Effect of enterohepatic circulation on plasma levels of [^{3}H]-*N*-(2 hydroxy ethyl) 2-phenyl ethyl carbamate. Animals were lightly anaesthetized with ether and their bile ducts ligated. The same procedure was carried out in another group of animals except that the bile ducts were not ligated (sham operated). The test substance, 12 mg/kg, was given 16 h later via the tail vein. Each point is the mean (±s.e.) of 7 determinations except those at 0.5 and 8 h which are means of 4 determinations. The *P* values for the means of total radioactivity of bile duct-ligated (▲) compared to those of sham operated animals (●) at 0.5, 1, 2, 4, 6 and 8 h are, $P > 0.50$; $P < 0.005$; $P < 0.15$; $P < 0.001$; $P < 0.001$ and $P < 0.001$ respectively. The *P* values for the means of unchanged drug of bile duct-ligated (△) compared with sham operated animals (○) are, $P > 0.05$; $P > 0.20$; $P < 0.025$; $P < 0.001$; $P < 0.001$ and $P < 0.001$ respectively.

excreted in the bile in rats. Since only a small fraction of the dose is excreted in the faeces (unpublished observation), it was suggested that this substance was involved in enterohepatic circulation. In an attempt to obtain information on whether enterohepatic cycling of the drug contributes to its prolonged plasma levels, the bile duct was ligated about 16 h before drug administration. In a preliminary study, it was observed that the effect of ether on the rate of elimination of the carbamate from the blood was apparent up to 6 h following ether anaesthesia (see also Fig. 1) but not after 16 hours. The animals were therefore used 16 h after bile duct ligation or sham operation (also performed under ether anaesthesia).

Bile duct ligation greatly shortened the plasma half-life (β-phase) of the carbamate. In sham operated rats the plasma levels of the drug remained almost constant but declined in animals with ligated bile ducts (Figure 2).

Plasma levels of the carbamate declined at about the same rate in both bile duct ligated and sham operated animals during the α-phase, which lasted up to 2 h after drug administration (Figure 2).

Effect of bile duct ligation on the metabolism of N-(2 hydroxy ethyl) 2-phenyl ethyl carbamate in isolated perfused rat liver

Cholestasis has been postulated to be associated with alteration in the cytochrome P 450 dependent microsomal biotransformation system (see Schaffner, Bacchin, Hutterer, Scharnbeck, Sarkozi, Denk & Popper, 1971). The possibility that bile duct ligation might have influenced the biotransformation of the carbamate by the liver was tested in the rat isolated perfused liver preparation. Livers were isolated under pentobarbitone anaesthesia 16 h after bile duct ligation or sham operation and perfused as described above. [^{3}H]-*N*-(2 hydroxy ethyl) 2-phenyl ethyl carbamate, 3 mg, was added to the perfusate reservoir and mixed with the aid of a magnetic stirrer. The bile duct was cannulated in all cases. After 3 h of perfusion of livers from both bile duct ligated and sham operated rats, the unchanged drug content of the perfusate had declined to about 2% of the total radioactivity (Table 1). The ratios of unchanged drug to total radioactivity in the perfusate of bile duct ligated rats did not differ significantly from those of the sham operated ones, indicating that bile duct ligation did not alter the rate of biotransformation of the drug by the liver (Table 1).

Bile duct ligation caused a pronounced distension of the duct and the bile which was collected during the first 60-90 min of perfusion was lighter in colour than the one from the 'sham operated liver'.

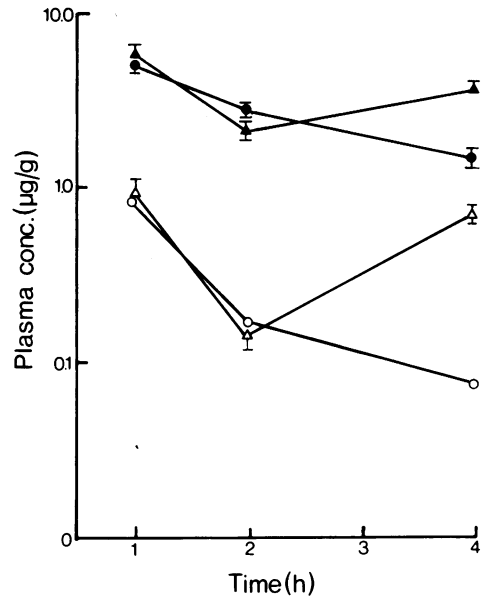

Fig. 3 Effect of ampicillin treatment on the plasma levels of [^{3}H]-*N*-(2 hydroxy ethyl) 2-phenyl ethyl carbamate. Animals were given ampicillin, 25 mg/rat by stomach tube in three divided doses on the first day and a single dose of 25 mg/animal on the second day. The carbamate, 12 mg/kg, was given via the tail vein about 2 h after the last dose of the antibiotic. Control animals were given saline instead of ampicillin. Each point is the mean of two determinations except that at 4 h which is a mean of three determinations. The *P* values for the means of total radioactivity at 1, 2 and 4 h are, $P > 0.40$; $P > 0.20$ and $P < 0.001$ respectively and for unchanged drug, $P > 0.40$; $P > 0.30$ and $P < 0.001$ respectively. (▲) total radioactivity of control animals; (●) ampicillin-treated animals; (△) unchanged drug of control rats; (○) ampicillin-treated rats.

Table 1 Effect of bile duct ligation on the rate of metabolism of *N*-(2 hydroxy ethyl) 2-phenyl ethyl carbamate in the rat

	Unchanged drug as % of total radioactivity after:					
Treatment	*5 min*	*15 min*	*30 min*	*60 min*	*120 min*	*180 min*
Bile duct ligated ($n = 4$)	85.75 ± 14.04	57.78 ± 6.34	35.52 ± 6.83	15.73 ± 3.34	4.035 ± 0.867	2.240 ± 0.495
Sham operated ($n = 3$)	72.49 ± 11.94	62.58 ± 6.54	37.39 ± 6.39	12.09 ± 4.18	3.967 ± 1.173	2.080 ± 0.652
P	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5

Sham-operated and bile duct-ligated animals were anaesthetized with pentobarbitone and their bile ducts cannulated. Livers were removed and perfused as described in the methods section. The carbamate (3 mg) was added to the perfusate reservoir and mixed by means of a magnetic stirrer. Perfusate samples were taken from the reservoir at various intervals and analysed for total radioactivity and unchanged drug as described in the methods section.

The values are expressed as % of total radioactivity ± s.e. mean.

Effect of ampicillin treatment on the plasma levels of N-*(2 hydroxy ethyl) 2-phenyl ethyl carbamate in rats*

The above results suggested that the conjugates of the carbamate and its metabolites initially secreted in the bile, were hydrolyzed and partially reabsorbed from the gastrointestinal tract, thus giving rise to enterohepatic circulation of the drug. To test this possibility, the effect of the antibiotic, ampicillin, on plasma levels of the carbamate was studied.

Ampicillin treatment had a profound effect on the plasma level of the drug. During the first 2 h following drug administration, the rate of decline of plasma levels of the carbamate was similar in both ampicillin-treated and control animals. Thereafter, the plasma levels of the unchanged drug rose from 0.146 μg/g at 2 h to 0.693 μg/g at 4 h in control animals. In the ampicillin-treated animals, they declined from 0.161 μg/g at 2 h to 0.072 μg/g at 4 h (Figure 3). The plasma levels of total radioactivity followed a similar pattern.

*Biliary excretion of [^{3}H]-*N*-(2 hydroxy ethyl) 2-phenyl ethyl carbamate*

About 50% of the given dose was excreted in the bile during 6 h, most of this being recovered during the first 90 minutes.

Hydrolysis of the bile with β-glucuronidase/arylsulphatase increased its unchanged drug content by more than forty-fold (Table 2).

Discussion

The present studies provide evidence that the prolonged half-life of *N*-(2 hydroxy ethyl) 2-phenyl ethyl carbamate in rats is largely due to enterohepatic circulation of the drug in this species. Pretreatment of the animals with the antibiotic, ampicillin, prior anaesthesia with ether or ligation of the bile duct, antagonized this phenomenon and markedly shortened the plasma half-life of the carbamate.

The gut flora are rich in enzymes that are capable of hydrolyzing conjugates (see Smith, 1966; Scheline, 1968). One of the most important of these enzymes, from the point of view of conjugate-hydrolysis in the gut, is β-glucuronidase. Very high activity of this enzyme has been found in the alimentary tract of many species, including the rat. Most of the enzyme activity is localized in the lower gut, particularly the caecum and colon (Marsh, Alexander & Levy, 1952). Although the conjugates of the carbamate have not been isolated, indirect evidence suggests that they are glucuronides and/or sulphates (Table 2). The conjugates of a compound excreted in the bile may undergo hydrolysis in the intestine, the liberated aglycones being absorbed to varying degrees and thereby establishing an enterohepatic circulation (Smith, 1966). The involvement of the carbamate in enterohepatic cycling can be explained in a similar way.

Most conjugates are polar acidic compounds, largely ionized at the pH prevailing in the intestine and probably would not be absorbed (Schanker, 1963). They then pass along the intestine and may be hydrolyzed in the lower intestine. Passage of a substance through the gastrointestinal tract in the rat may take several hours (Dobbs, Hall & Steiger, 1970). Since the proposed sites of conjugate-hydrolysis of the carbamate are in the lower gut, it is probable that the delay of about 2 h before this compound and its metabolites cleared in the bile are reabsorbed, is due to the time required for the conjugates to reach these sites (see Figures 1, 2 and 3).

Ligation of the bile duct inhibits excretion of the drug and its metabolites (mainly in form of conjugates) via the bile into the duodenum. This prevents access of the conjugates to the lower intestine (sites of hydrolysis) and thus establishment of enterohepatic circulation.

Ampicillin-treatment greatly reduces the β-glucuronidase activity of caecal contents in rats (Dobbs *et al.*, 1970). This effect may be an important factor in the antagonism of enterohepatic circulation of the carbamate by ampicillin in the rat. However, ampicillin treatment also appears to retard the speed of propulsion of gut contents through the gastrointestinal tract (Dobbs *et al.*, 1970). Retardation of the passage of the carbamate (and its metabolites) conjugates to the sites in the lower intestine where they could be hydrolyzed will delay the onset of enterohepatic circulation, thus leading to an effect similar to that produced by direct inhibition of the enzymes, at least during the early intervals following drug

Table 2 Biliary excretion of [^{3}H]-*N*-(2-hydroxy ethyl) 2-phenyl ethyl carbamate in rats following intravenous administration of 12 mg/kg

Unchanged drug + metabolites (total radioactivity)	*Unchanged drug before enzyme hydrolysis*	*Unchanged drug after enzyme hydrolysis*
50.3 ± 3.3	0.16 ± 0.01	6.76 ± 0.26

The values are expressed as percent of the given dose. Bile was collected during 6 h following drug administration. (n = 2).

administration. The relative roles played by these factors on the ampicillin-induced inhibition of enterohepatic circulation are not apparent from the present studies.

Ether is perhaps the most widely used anaesthetic agent in common laboratory animals when short-lasting anaesthesia is desired. Very often the possibility that ether may still be exerting important effects long after the animals have regained full consciousness is not taken into consideration. The present studies show that ether can exert a profound effect on the pharmacokinetics of the carbamate (Figure 1). This action is apparent for at least 6 h from the time the animals regain full consciousness from ether anaesthesia. The effect can be brought about by (a) inhibition of the biliary excretion of the compound and its metabolites, (b) inhibition of gastrointestinal activity leading to delayed arrival of the conjugates to the site of hydrolysis, (c) inhibition of the enzymes which hydrolyze the conjugates in the lower part of the intestine or a combination of these factors. The observations that ether anaesthesia inhibits biliary excretion of the carbamate in the rat (Obianwu & Eklund, 1973) and that this anaesthetic agent considerably inhibits gastrointestinal activity (Goodman & Gilman, 1970) are in accord with the above views.

Cholestasis induced by ligation of the bile duct has been reported to inhibit the miscrosomal biotransformation of different substances (see Schaffner *et al.*, 1971). This effect is related to the duration of the cholestasis and is absent or very slight during the first 24 h of bile ligation. In any case, bile duct ligation did not appear to influence the biotransformation of the carbamate by the isolated perfused rat liver (Table 1). Bile duct ligation may be a valid and convenient method of studying enterohepatic circulation in the rat, provided cholestasis does not exceed 24 hours.

One of the important consequences of a substance involved in enterohepatic circulation is that the substance may persist in the body for unduly long periods and the hepatobiliary tract may be subjected to concentrations of the substance much higher than those of the blood. Since the enterohepatic circulation of many compounds is species dependent (Smith, 1966), the toxic effects and the pharmacokinetics of such compounds may therefore be species dependent. In view of this, it may be important to test any compound excreted in significant amounts in the bile (greater than 10% of the given dose) for involvement in enterohepatic circulation. Information so obtained may be of great value in evaluating new compounds.

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