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Effects of diethyl ether anaesthesia on the pharmacokinetics of antipyrine and paracetamol in the rat

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The effects of two anaesthetic procedures: continuous administration of ether throughout the periods of drug infusion and blood sampling, and brief ether administration (5 min) before drug infusion were examined. Continuous ether reduced total clearance, β, and k_{10} for both drugs, and in addition V_c and V_β for antipyrine. Brief ether anaesthesia had no effect on antipyrine kinetics, but caused a decrease in total clearance, k_{10} and V_β of paracetamol. The rates of distribution and redistribution of the drugs were unchanged by ether. The data suggest that ether interferes with the hepatic conjugation of paracetamol and may interfere with the hepatic oxidation of antipyrine and therefore should not be used as an anaesthetic when the kinetics of drugs with short elimination half-lives are studied. It may be used for drugs with longer half-lives.

Diethyl ether is widely used for anaesthesia in animal experiments. Although an early report by Baekeland & Greene (1958) suggested that pentobarbitone was eliminated at a reduced rate in rats under ether anaesthesia, the possible shortcomings of this agent in studies of drug metabolism and disposition have received only limited attention (Umeda & Inaba 1978). Investigators in this field who use diethyl ether usually describe 'a brief course of ether anaesthesia' assuming that the exposure to ether is too short to have any significant effect on drug metabolism or drug kinetics.

We have investigated the pharmacokinetics of drugs eliminated via different metabolic pathways in rats anaesthetized with diethyl ether. Two procedures of anaesthesia were used: A brief course of anaesthesia, and continuous anaesthesia during the period of drug administration and blood sampling.

MATERIALS AND METHODS

Chemicals

The chemicals were purchased from the following sources: antipyrine, paracetamol, heparin, diethyl ether and fluanison/fentanyl (Hypnorm) from Norsk Medisinaldepot, Oslo, Norway; antipyrine-[*N*-methyl-[^{14}C] and [3H] paracetamol, generally labelled from New England Nuclear, Boston, Mass., U.S.A.

Animals and operations

Male Wistar rats, 200–300 g were used. Under fluanison fentanyl anaesthesia (6·6/0·13 mg kg^{-1} s.c.) the inguinal artery and vein were cannulated with PE 50 tubing previously filled with heparinized 0·9% NaCl (saline), the indwelling part of the tubing having been stretched to reduce its diameter and lubricated with silicone oil to facilitate insertion. The cannula was secured and the tubing was transferred dorsally through a subcutaneous tunnel and made accessible through a skin perforation in the lumbal region. The rats were placed in restraining cages and allowed free access to food and water.

Experiments

Since the object was to study effects of routine procedures for ether anaesthesia, standardization was aimed at the clinical level of anaesthesia and not at the dose of ether. The rats were placed in a 2 litre airtight chamber containing filter paper soaked with ether. Immediately after the loss of righting reflexes the rats were transferred to a restraining cage where ether was administered by covering the nose with a beaker containing a soaked cotton plug, sufficient ether being administered to prevent the return of the righting reflex during anaesthesia.

In one set of experiments ether was given continuously throughout the periods of drug infusion and blood sampling, and the effects of ether anaesthesia were identified by comparison with a control group. Each rat was given antipyrine on only one occasion.

In the second set of experiments each rat was given the test drug on two occasions. First the control experiments were carried out, i.e. drug was infused without anaesthesia, and blood samples were taken (see below). After an interval of more than 5 average

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elimination half-lives following the first drug infusion, ether anaesthesia was given as described above, and ether administration was discontinued when drug infusion was started. In these experiments the total exposure time to ether was typically about 5 min, and it never exceeded 10 min. In parallel with these experiments identical experiments were carried out with rats that received no ether on either the first or the second occasion. This design was chosen because the validity of a cross-over design might be limited by possible residual effects of ether.

The night before the experiments the cannulas were placed in the inguinal artery and vein. At 8 a.m. control pharmacokinetics of all animals were determined and at 10 a.m. (paracetamol) or 3 p.m. (antipyrine) the kinetic studies were repeated either without ether (control) or with ether. Before the second infusion of the test drug, a blood sample was drawn to check that the preceding dose had been eliminated.

The test drugs were infused via the cannula in the inguinal vein dissolved in a volume of 0·6–0·9 ml saline. The infusion time was 30 s and was immediately followed by flushing of the cannula with 0·5 ml saline. The same dose was used for [^{14}C]-antipyrine and [^{3}H]paracetamol: 15 mg kg^{-1}, 1–2 μCi/animal. Blood samples were drawn from the cannula in the inguinal artery 3, 6, 9, 15, 20, 30, 40, 60, 90, 120, 150 and 180 min after dosing with antipyrine and 3, 6, 9, 12, 15, 20, 30, 40, 50 and 60 min after dosing with paracetamol. Each time, 0·1 ml of blood was removed.

Analytical methods

Concentrations of antipyrine and paracetamol were measured in whole blood essentially by the extraction methods of Bakke et al (1974) and Cohen et al (1974).

Calculations

The data were analysed according to a two compartment open model with first order elimination kinetics (Gibaldi & Perrier 1975) .Total clearance was calculated by Dose/ ($A/_{\alpha}$ + $B/_{\beta}$), where A and B are y-intercepts of the extrapolated lines of the α phase and β phase, respectively. V_C was calculated by dividing the dose by (A + B). V_{β} was obtained by dividing total clearance by β.

The Wilcoxon test for two samples was used for statistical analysis of the experiment with continuous ether. The data of the brief ether experiment were analysed by two-way analysis of variance (Sokal & Rohlf 1973). This method permits simultaneous analysis of the four data groups for significant differences between animal groups and differences between observations at different times after operation. Significant interaction was taken to indicate that the experimental group developed differently from the control group, i.e. effect of ether anaesthesia.

Only when interaction was non-significant, was the test extended to analysis of differences between groups and times. *P*-values less than 0·05 were taken to indicate statistical significance in two-tailed testing. When one datum point in a group was missing, corresponding data points in the remaining groups were deleted to obtain uniform size of the data groups. Otherwise a two-way analysis of variance would be impossible.

RESULTS

The plasma concentration vs time curves of antipyrine and paracetamol were significantly changed after continuous ether administration (Figs 1, 2). Continuous ether anaesthesia caused a significant decrease of k_{10}, β, and Cl and an increase of $t_{\frac{1}{2}}$ (β) for antipyrine and paracetamol ($P < 0{\cdot}05$). In addition, there was a decrease of V_C and V_{β} for antipyrine.

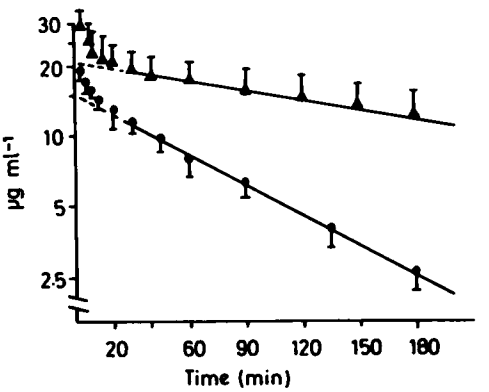

FIG. 1. Plasma concentrations of antipyrine after 15 mg kg^{-1} i.v. during continuous ether anaesthesia (▲) and in controls (●). Values are mean with s.d. n = 6 in each group.

Brief ether anaesthesia caused no significant changes in antipyrine kinetics (Table 1). There was a significant difference between the animal groups in elimination kinetics of the β-phase, and the group with the higher body weight showed longer half-life and lower total clearance. The volumes of distribution V_C and V_{β} diminished with time after operation. After brief ether anaesthesia V_{β}, total clearance and k_{10} of paracetamol were reduced significantly (Table 2). V_C diminished with time after operation.

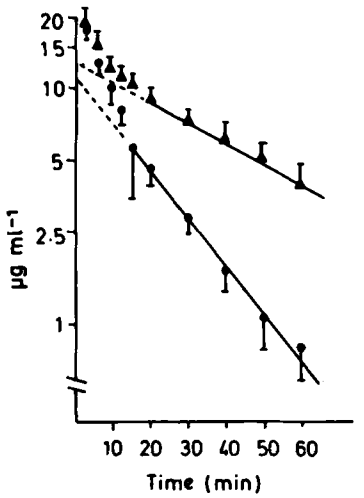

FIG. 2. Plasma concentrations of paracetamol after 15 mg kg^{-1} i.v. during continuous ether anaesthesia (▲) and in controls (●). Values are mean with s.d. n = 6 in each group.

DISCUSSION

Continuous ether anaesthesia caused substantial reductions in the elimination rates of antipyrine and paracetamol. Since both drugs are eliminated by hepatic metabolism, the results suggest important effects of ether on liver function. However, additional data from in vitro or supplementary in vivo studies are usually needed when pharmacokinetic parameters are interpreted in terms of biochemical and physiological functions (Vesell 1979). The metabolism of antipyrine and paracetamol was decreased in suspensions of isolated hepatocytes when diethyl ether was added to the medium (Aune & Mørland 1980). Ether also inhibits drug metabolizing enzymes in liver homogenates (Baekeland & Greene 1958; Hempel et al 1975). Since antipyrine is eliminated by microsomal oxidation (Aarbakke 1978) and paracetamol is eliminated by microsomal glucuronidation and cytosolar sulphatation (Thomas et al 1974) in the rat, the results suggest that drug metabolizing enzymes of different types and cellular localization are affected by diethyl ether.

Brief ether anaesthesia before drug infusion caused significant changes in the kinetics of paracetamol, but not of antipyrine. Grossly similar inhibition of antipyrine and paracetamol metabolism has been found in hepatocytes incubated with ether from 2·5 to 30 mM (H. Aune, personal communication). Hence, differences in the sensitivity of the liver enzymes to the action of ether is not a likely explanation of the different response in elimination kinetics of the drugs. Alternatively, the different rates of elimination of paracetamol and antipyrine could explain the observation.

Typical values of $t_{\frac{1}{2}}$ (β) of antipyrine and paracetamol are 75 and 15 min, respectively. Because of its slow elimination, ether can be expected to remain in the liver in inhibitory concentrations for several control half-lives of paracetamol (Goldstein et al 1974). With antipyrine most of the ether will be eliminated within its first half-life. Consequently brief ether seems to be unsuitable as an anaesthetic for pharmacokinetic experiments on drugs with a short half-life (e.g. paracetamol), but may be used for studies of drugs with long half-lives (e.g. antipyrine).

Table 1. Effects of brief ether anaesthesia (5 min) on the pharmacokinetics of antipyrine in rats. Antipyrine (15 mg kg^{-1}) was dosed on two occasions to each animal with an interval >5 $t_{\frac{1}{2}}$ (β). The drug was infused via a cannula in an inguinal vein, and blood was drawn from a cannula in an inguinal artery. The experimental group was given ether at 3 p.m. Values are mean (with s.d.).

Kinetic variable	Control rats (n = 6)[a]		Experimental rats (n = 6)[a]	
	8 a.m.	3 p.m.	8 a.m.	3 p.m.
α (10^{-2} min^{-1})	11·8 (2·2)	13·7 (3·7)	12·1 (8·5)	16·6 (6·8)
β (10^{-2} min^{-1})	9·5 (1·1)	9·4 (1·2)	8·2 (1·4)	7·1 (0·9)
$t_{\frac{1}{2}}(\alpha)$ (min)	6·0 (1·3)	5·4 (1·5)	7·8 (4·1)	4·9 (2·4)
$t_{\frac{1}{2}}(\beta)$ (min)[b]	73·9 (8·0)	73·9 (8·2)	86·7 (14·9)	98·5 (12·8)
V_c (litre kg^{-1})[c]	0·64 (0·11)	0·54 (0·05)	0·66 (0·15)	0·48 (0·12)
$V\beta$ (litre kg^{-1})[c]	0·99 (0·2)	0·82 (0·12)	1·02 (0·26)	0·79 (0·09)
Cl_T (ml min^{-1} kg^{-1})[b,c]	9·4 (1·8)	7·6 (1·7)	8·2 (1·5)	5·6 (0·8)
k_{12} (10^{-2} min^{-1})	3·8 (1·0)	4·4 (1·6)	4·3 (3·9)	6·3 (4·5)
k_{21} (10^{-2} min^{-1})	7·4 (2·2)	8·8 (2·5)	7·2 (4·2)	9·8 (4·4)
k_{10} (10^{-2} min^{-1})	15·9 (3·2)	15·2 (1·7)	13·0 (2·4)	12·9 (4·1)

a = body weight: control rats 242 ± 20 g, experimental rats 290 ± 24 g.
The data were analysed by means of two-way analysis of variance:
b = significant difference between groups.
c = significant difference between times.

Table 2. Effects of brief ether anaesthesia (5 min) on the pharmacokinetics of paracetamol in rats. Paracetamol (15 mg kg^{-1}) was dosed on two occasions to each animal with an interval > 5 $t_{\frac{1}{2}}$ (β). The drug was infused via a cannula in an inguinal vein and blood was drawn from a cannula in an inguinal artery. The experimental group was given ether at 10 a.m. Values are mean (with s.d.).

Kinetic variable	Control rats (n = 6)[a]		Experimental rats (n = 6)[a]	
	8 a.m.	10 a.m.	8 a.m.	10 a.m.
α (10^{-2} min^{-1})	19·2 (2·9)	21·1 (5·1)	17·6 (2·0)	17·4 (8·7)
β (10^{-2} min^{-1})	46·9 (5·7)	44·4 (4·5)	52·2 (10·1)	46·0 (6·8)
$t_{\frac{1}{2}}(\alpha)$ (min)	3·7 (0·6)	3·5 (0·9)	4·0 (0·5)	5·2 (3·2)
$t_{\frac{1}{2}}(\beta)$ (min)	15·0 (1·8)	15·8 (1·4)	13·7 (2·8)	15·3 (2·1)
V_c (litre kg^{-1})[b]	0·57 (0·05)	0·46 (0·08)	0·62 (0·04)	0·52 (0·07)
$V\beta$ (litre kg^{-1})[c]	0·97 (0·12)	0·98 (0·04)	1·09 (0·13)	0·72 (0·12)
Cl_T (ml min^{-1} kg^{-1})[c]	44·3 (2·8)	41·9 (0·7)	58·8 (16·4)	33·1 (7·0)
k_{12} (10^{-2} min^{-1})	4·7 (1·9)	6·4 (3·0)	3·7 (0·3)	4·1 (3·5)
k_{21} (10^{-2} min^{-1})	11·1 (1·7)	11·2 (4·2)	10·1 (2·4)	11·0 (3·7)
k_{10} (10^{-2} min^{-1})[c]	78·3 (6·6)	93·7 (18)	88·4 (9·2)	64·7 (13·1)

a = body weight: Control rats 293 ± 16 g, experimental rats 295 ± 14 g.
The data were analysed by means of two-way analysis of variance:
b = significant differences between times.
c = significant interaction, i.e. ether effect.

The mechanisms of altered distribution of the drugs under ether anaesthesia remain speculative. Ether is known to change the haemodynamic parameters of several species (Su et al 1979; Walsh & Ferrone 1979), but minor changes have been found in the fraction of cardiac output reaching the different organs with and without ether (Su et al 1979). Similarly, the plasma/brain ratio of pentobarbitone was unaffected by ether administration (Baekeland & Greene 1958).

In view of the present data, alternative methods of animal anaesthesia for studies of drug kinetics should be investigated.

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