International Journal of Pharmaceutics, 38 (1987) 261–263
Elsevier

IJP 01302

Urethane anaesthesia: altered pharmacokinetics of the renally eliminated organic anion *p*-aminohippuric acid

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(Received 18 March 1987)
(Accepted 31 March 1987)

Key words: Urethane; Anaesthetic; *p*-Aminohippuric acid; Pharmacokinetics; Rat; Pentobarbitone

The assessment of a compound's pharmacokinetic characteristics is often conducted in small laboratory animals and in order to minimize pain and discomfort, such assessments are usually performed under anaesthesia. The administration of an anaesthetic agent and the effect this may have on the disposition of the compound under investigation is an important case for consideration. However, there is little information about the possible effects that an administered anaesthetic may have on the pharmacokinetic profile of a xenobiotic. However, alterations in the disposition of thiamine (Pipkin and Stella, 1982) carboxyflourescein (Woolfrey et al., 1985) and diphenylhydantoin (Umeda and Inaba, 1978) by various anaesthetic agents have previously been noted.

The present study has investigated the effects of 3 non-gaseous anaesthetic agents on the pharmacokinetics of *p*-aminohippuric acid (PAH). The elimination of PAH is independent of metabolic transformation. It is not taken up by the kidney and only 10% is bound to plasma proteins and its clearance and extraction ratio are often used to evaluate renal blood flow. Use of PAH as a tool compound allows the study of the selective influence of anaesthetics on renal mechanisms of excretion. This present study was performed in male Wistar rats (250 ± 16 g) and the 3 anaesthetic regimens studied were:

(a) Fentanyl and fluanisone mixture ('Hypnorm' Janssen), (0.26 and 8.3 mg/kg i.p., respectively) given in combination with midazolam ('Hypnovel', Roche), (4.16 mg/kg i.p.) for induction of anaesthesia. Fentanyl and fluanisone (0.08 and 2.50 mg/kg i.p.) were subsequently given every 30 min for maintenance.

(b) Urethane (Sigma) (1.75 g/kg i.p.) given as a 25% w/v solution in normal saline.

(c) Pentobarbitone sodium (Sigma) (67 mg/kg i.p.) given as a 1.5% w/v solution in normal saline for induction. Pentobarbitone sodium (6–7 mg/kg i.p.) given subsequently every 60 min as a maintenance dose.

The anaesthetic doses used in this study were the minimum required for surgical anaesthesia and are within the dose ranges commonly employed for laboratory anaesthesia in the rat (Green, 1979).

When the animals had reached a sufficient depth of anaesthesia, as judged by corneal reflex test and response to painful stimuli, the left common jugular vein was exposed and catheterised (Portex polythene tubing PP50) to allow a single i.v. injection of 5×10^{-3} μmol (2.5 μCi)/250 g animal of ^{3}H-labelled PAH (Amersham, radio-

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chemical purity 98.2%) in normal saline (approximate volume 250 μl) and to allow i.v. normal saline fluid replacement throughout the course of the experiment. Caudal vein catheterisation (Portex polythene tubing PP25), was performed to allow the collection of blood samples (100 μl) into heparinised tubes. All surgical wounds were covered with gauze kept moist with normal saline to minimize tissue fluid loss. Rectal temperatures were monitored and maintained at $38 \pm 1°$ C using an incandescent lamp and heated surgical tray. Tracheotomies were performed as an aid to respiration throughout the period of anaesthesia.

The blood samples prior to radiochemical analysis were solubilised and decolourised, the scintillation fluid being a mixture of 0.5 N HCl/Instagel (Packard), 1 : 9 (v/v). All samples were analysed against a blank control using non-radioactive blood on a LKB 1217 Rackbeta liquid scintillation counter. Results from each animal were analysed by non-compartmental pharmacokinetics and according to a two-compartment model using a non-linear, least-squares regression programme (Holford, 1983) (Table 1). There were no significant ($P > 0.05$) differences between 'Hypnorm'/'Hypnovel' and pentobarbitone sodium anaesthetised groups for any of the pharmacokinetic parameters investigated. However, in urethane anaesthetised rats the results demonstrate a significant ($P < 0.05$) lowering, to almost a half, in the total body clearance, calculated from blood (Cl), of PAH with a 50% elevation in the area under the blood concentration–time curve, from time 0 to infinity (AUC) and a two-fold prolongation in elimination half-life ($t_{\frac{1}{2}\beta}$) without any significant ($P > 0.05$) alteration in the volumes of distribution ($V_{d_{ss}}$ and V_{d_β}) when compared with both 'Hypnorm'/'Hypnovel' and pentobarbitone sodium anaesthetised groups. The influence of pentobarbitone anaesthesia on the total body clearance of PAH, calculated in this study, is in close agreement with previously obtained values of 5.20 ml/min/100 g body wt. in rats anaesthetised with pentobarbitone 40 mg/kg i.v., a PAH clearance of 6.95 ml/min/100 g body wt. being obtained in unanaesthetised animals (Walker et al., 1983.). The difference in PAH elimination half-life between the anaesthetic agents used in this study appears not to be a reflection of an alteration in the volume of distribution but rather to a change in the total body clearance, which for PAH must reflect an alteration in renal elimination mechanisms, an alteration likely mediated via renal haemodynamic mechanisms.

It is well established that barbiturate anaesthesia results in impairment of renal blood flow compared to the unanaesthetised animal (Koeppen et al., 1979; Linas et al., 1980; Burger et al., 1976), an alteration which is associated with activation of the renin–angiotensin system (Walker et al., 1986)

Autoregulation of renal blood flow in rats, below a mean arterial pressure of 95 mm Hg has been reported to have a low degree of efficiency (Arendshorst et al., 1975). Significantly lower mean arterial pressures have previously been reported in rats anaesthetised with urethane, compared to pentobarbitone anaesthetised animals (Armstrong et al., 1982), significant urethane-induced reductions in diastolic blood pressure (Buelke-Sam et

TABLE 1

Pharmacokinetic parameters of para-aminohippuric acid and the influence of anaesthetic agents

Anaesthetic/ regimens	Cl_{blood} (ml/min/250 g)	AUC (dpm **/μl · min^{-1})	$V_{d_{ss}}$ (ml/250 g)	V_{d_β} (ml/250 g)	$t_{\frac{1}{2}\beta}$ (min^{-1})	Hybrid rate constants	
						α (min^{-1})	β (min^{-1})
Hypnorm/Hypnovel	8.94 ± 1.18	643 ± 92	287 ± 42	376 ± 97	31 ± 7	0.133 ± 0.046	0.025 ± 0.006
Urethane	5.69 ± 0.85 *	931 ± 58 *	360 ± 39	456 ± 26	56 ± 10 *	0.191 ± 0.045	0.010 ± 0.006 *
Pentobarbitone	10.53 ± 1.79	564 ± 99	329 ± 34	442 ± 42	29 ± 3	0.113 ± 0.037	0.024 ± 0.002

* $P < 0.05$. Statistically different from all other treatments. One-way analysis of variance and Duncan's multiple range test. Results Mean $\pm$ SD ($n = 5$).

* *dpm = decompositions per minute of ^{3}H-labelled isotope.

al., 1978) being indicative of an increase in peripheral vascular resistance. Pettinger et al. (1975) has reported that in urethane- compared to pentobarbitone-anaesthetised rats an elevation in plasma renin activity is observed, which was independent of surgery and blood sampling. Urethane anaesthesia, in chronically catheterised rats has been reported to produce significant elevations in plasma adrenaline and noradrenaline concentrations compared to the conscious control group (Carruba et al., 1987). In urethane- compared to pentobarbitone-anaesthetised rats, significantly higher plasma adrenaline levels have been observed, in the intact but not adrenalectomised or pithed animal (Armstrong et al., 1982). Such elevations in circulating catecholamines, which in their own right can have a deleterious effect on renal haemodynamics (Johns, 1979), can stimulate renin secretion via adrenergic receptors at the juxtaglomerular apparatus (Reid et al., 1978).

Pettinger et al. (1975.) have reported the effects of a variety of anaesthetic agents on renin secretion, their differential effects may well be important in dispositional studies, performed under anaesthesia and involving renally eliminated compounds.

To conclude, this work has demonstrated that different anaesthetic agents may dramatically alter the pharmacokinetics of PAH, and that urethane anaesthesia may have a more pronounced influence on the renal elimination of compounds compared to other anaesthetic regimens. Consideration must be given to the effects of anaesthetic agents on renal elimination mechanisms when pharmacokinetic studies are to be performed in animals under anaesthesia. The 'Hypnorm'/'Hypnovel' anaesthetic combination may provide a superior alternative to urethane anaesthesia if the use of pentobarbitone is contra-indicated.

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