Drug Metabolism and Laboratory Anesthetic Protocols in the Rat: Examination of Antipyrine Pharmacokinetics

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INTRODUCTION

The use of an anesthetic regimen is invariably a component of laboratory pharmacokinetic studies performed in the rat, enabling the surgical catheterization of vascular tissue. The two commonly used surgical protocols involve the use of (a) the acute surgically prepared anesthetized animal, where the pharmacokinetic study is performed under anesthesia immediately upon completion of surgical catheterization, and (b) the conscious chronically catheterized animal, where upon completion of surgical catheterization, a period of recovery time is allowed prior to the performance of the pharmacokinetic study in the conscious animal. Limited attention has been given to the possible differential influence that these variables (i.e., anesthetic regimen and surgical protocol) may have upon laboratory drug disposition studies.

Recent reports (1,2) have demonstrated that the laboratory anesthetic urethane can have a profound influence upon renal hemodynamics in the rat and upon the subsequent clearance of compounds eliminated primarily by the renal route: gentamicin (2), *p*-aminohippurate (3), thiamine (4), and carboxyfluorescein (5). In these recent reports comparisons of xenobiotic disposition between the conscious chronically catheterized and the acute surgically prepared anesthetized animal were also made (2).

The objective of the present investigation was to examine the influence of some commonly used laboratory anesthetic protocols upon drug metabolism in the rat. The experiments were designed to investigate the concomitant effects of both the anesthetic regimen and the surgical protocol adopted, rather than the effects of anesthesia in isolation.

The disposition of antipyrine was chosen to assess intrinsic hepatic drug metabolism. Antipyrine is almost exclusively metabolized by the hepatic microsomal mixed-function oxidase system, with its metabolism mediated by at least three isozymes of cytochrome P450, which as yet have not been identified (6). Antipyrine exhibits low extents of protein binding, ≈13% (7), and a low hepatic extraction ratio, ≈0.01 (8), properties which allow antipyrine pharmacokinetics to be interpreted in terms of intrinsic clearance, essentially exclusive of protein binding and hepatic blood flow considerations.

MATERIALS AND METHODS

This study was conducted in male Sprague–Dawley rats (n = 6 for each experimental group) (Bantin and Kingman, Fremont CA), weighing (mean ± SD) 263 ± 18 g.

Acute Surgically Prepared Anesthetized Animals. The anesthetic regimens consisted of the following:

(a) Pentobarbital sodium (Sigma Chemical Co., St. Louis, MO), 60 mg/kg i.p., was used for induction of anesthesia, with subsequent maintenance doses of 6 mg/kg i.p. administered every 50 min.

(b) Urethane (Sigma Chemical Co.), 1.6 g/kg i.p., was given as a single dose.

(c) Ketamine (Vetalar; Parke-Davis, Division Warner-Lambert Co., Morris Plains, NJ), 80 mg/kg i.p., was given in combination with xylazine (Gemini; Rugby Laboratories Inc., Rockville Centre, NJ), 13 mg/kg i.m., for induction of anesthesia. Ketamine (15 mg/kg i.p.) was subsequently administered every 50 min for maintenance.

(d) A fentanyl and droperidol mixture (Innorvar-Vet; Pitman-Moore, Mundelein, IL), 0.08 + 4.0 mg/kg, respectively, was given i.p., followed immediately by Valium (Elkins-Sim Inc., Cherry Hill, NJ), 5 mg/kg i.m., for induction of anesthesia. Anesthesia was maintained by fentanyl and droperidol (0.02 + 1 mg/kg, respectively) administered i.p. every 40 min.

The anesthetic doses used in this study were the minimum required to produce surgical anesthesia and are within the dose ranges commonly employed for laboratory anesthesia in the rat (9,10).

Once the animals had reached a sufficient depth of anesthesia, the left jugular vein and right carotid artery were catheterized as previously described (2). To ensure against the consequences associated with anesthetic-induced hypothermia (9), such as alterations in peripheral hemodynamics and in drug metabolism, rectal temperatures were monitored and maintained at 38 ± 1°C. The pharmacokinetic study began immediately following completion of the surgical catheterization.

Chronically Catheterized Conscious Animals. To enable implantation of jugular vein and carotid artery catheters, the animals were anesthetized with anesthetic-grade ethyl ether (Fisher Scientific, Springfield, NJ) for a period not exceeding 15 min. The jugular vein and carotid artery were catheterized as described previously (2). The pharmacokinetic study began 24 hr following completion of the surgical catheterization.

Individual animals from each of the four anesthetized experimental groups were studied concurrently over an 18-day period. Individual animals comprising the chronically catheterized conscious group were not studied on the same day as anesthetized animals but were studied randomly throughout this period. All animal experiments began with the i.v. injection of antipyrine at 12 noon ± 1 hr.

Antipyrine Disposition. Antipyrine (20 mg kg^{-1}; Aldrich Chemical Co., Inc., Milwaukee, WI) dissolved in 0.9%

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saline was injected into the left jugular vein. Eleven carotid artery blood samples (250 μl) were collected over a 3.5-hr period with i.v. saline fluid replacement. Antipyrine plasma concentrations were analyzed by a HPLC assay based upon a previously published method (11). Briefly, plasma samples (100 μl) were made alkaline with 50 μl of 1.0 *M* NaOH, to which the internal standard, 50 μl of phenacetin (10 μg/ml) (Aldrich Chemical Co., Inc.), was added. The samples were extracted twice with 5 ml of pentane/dichloromethane (6:4). Recoveries for both antipyrine and phenacetin were >90%. The HPLC system consisted of an ultraviolet detector (254 nm) and a mobile phase composed of 20% acetonitrile/0.01 *M* phosphate buffer (pH 4.5), pumped isocratically at a flow rate of 1 ml/min through a C_{18} column (Beckman Instruments., Inc, Altex Division, San Ramon, CA). Under these conditions, no interfering peaks result from the metabolites of antipyrine. The anesthetic agents were tested and found not to interfere with the assay. Inter- and intraday precision analysis yielded coefficients of variation of <8% at 0.25 μg/ml and <1.5% at 30 μg/ml. Accuracy was to within 5.4 and 1.8% at 0.25 and 30 μg/ml, respectively.

Pharmacokinetic and Statistical Analysis. A one-compartment model was fitted to the antipyrine plasma concentration–time data using a nonlinear least-squares regression program (SIPHAR; Simed, Creteil, France) (weighting factor $1/y^2_{observed}$). The predicted rate constant (k) was used in the calculation of elimination half-life ($T_{1/2}$). Other pharmacokinetic calculations included volume of distribution [$V_{area} = Dose_{i.v.}/(k \cdot AUC)$] and clearance [$CL = Dose_{i.v.}/AUC$]. The area under the plasma concentration–time curve from time zero to infinity (AUC) was calculated using the log trapezoidal method, with the area t_{last} to infinity calculated as C_{last}/k.

Statistical analysis was performed using one-way analysis of variance and Duncan's multiple-range test (12). Results are statistically different at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

The results in Table I demonstrate differential effects of our laboratory protocols upon the clearance and half-life of antipyrine. Ketamine/xyalzine clearly yielded the lowest clearance and longest half-life. Innorvar-Vet was the only anesthetic procedure which showed no differences from the chronically catheterized conscious animal. Antipyrine clearance in the conscious group is comparable to antipyrine clearance values previously reported for rats treated similarly by others (13). No statistically significant ($P > 0.05$) difference between the experimental groups was observed in antipyrine volume of distribution.

Given the lipophilic nature of anesthetic agents, and the fact that many of these compounds are metabolized by the microsomal mixed-function oxidase system, it is not inconceivable that some anesthetics may interact with the membranes of the hepatic endoplasmic reticulum and influence the metabolism of other drugs. An inhibitory effect upon microsomal metabolism following the acute exposure to the volatile anesthetics diethyl ether (14) and halothane (15) is recognized. Effects of certain halogenated anesthetic agents (i.e., halothane, isoflurane, and enflurane) upon glucuronidation have been observed (16), with marked anesthetic-induced reductions in hepatic uridine diphosphoglucuronic acid. In the present study all the acute surgically prepared anesthetized protocols resulted in antipyrine clearance values lower than that observed in the conscious chronically catheterized group. Antipyrine clearance in the pentobarbital, urethane, and ketamine/xylazine protocols was significantly ($P < 0.05$) reduced, to 83, 75, and 52%, respectively, of the value observed in conscious animals. Of significance in the comparison between conscious and anesthetized groups, beyond anesthesia per se, is the animals' response to the different surgical protocols. In a report by Chindavijak *et al.* (17), the presence of indwelling venous catheters in the conscious chronically catheterized rat for a period of 48 hr results in a reduction in the intrinsic clearance of antipyrine to 80% of the value observed at 2 hr after catheter implantation.

Reports examining the influence that injectable laboratory anesthetic agents may have upon cytochrome-P450-mediated drug metabolism are limited. However, in an *in vitro* rat hepatic microsome preparation, pentobarbital has been reported to act as an inhibitor of tolbutamide hydroxylation (18), and to inhibit the clearance of phenytoin within an *in situ* perfused rat liver preparation (19). Ether inhalation anesthesia in the rat has been reported to have a more depressive effect upon the metabolism of theophylline (20) and phenytoin (21) than the injectable anesthetic urethane.

Urethane anesthesia in the rat results in considerable reductions in hepatic blood flow (≈50%) compared to the other injectable anesthetic agents (1), and thus hepatic oxygen delivery may be effected. The rate of oxygen delivery to the isolated perfused rat liver has been shown to influence

Table I. Antipyrine Pharmacokinetic Parameters in Conscious and Anesthetized Rats[a]

	P	U	K	I	C	Statistical comparisons[b]
CL (ml/min/kg)	5.15 ± 0.77	4.65 ± 0.71	3.20 ± 0.42	5.42 ± 0.92	6.21 ± 0.59	C I P U K
V_{area} (ml/kg)	701 ± 25	723 ± 20	717 ± 32	718 ± 39	691 ± 35	U I K P C
$T_{1/2}$ (min)	96 ± 18	110 ± 13	157 ± 20	93 ± 10	78 ± 10	K U P I C

[a] Results presented as mean ± SD; $n = 6$ for each experimental group. P, pentobarbital anesthetized; U, urethane anesthetized; K, ketamine/xylazine anesthetized; I, Innorvar-Vet anesthetized; C, conscious.

[b] Groups are arranged left to right in the order of descending magnitude for each pharmacokinetic parameter. Groups jointly underlined are not significantly different ($P > 0.05$) from each other. Statistics for Duncan's multiple-range test: CL, mean square error (MSE) 0.493; V_{area}, MSE 952; $T_{1/2}$, MSE 215.

significantly the intrinsic clearance of antipyrine (22). Of further consideration to urethane anesthesia and hepatic function may be the demonstration that an i.p. injection of urethane in the rat is associated with the development of intraabdominal toxicity and necrosis of the liver (23).

The appreciable effect of the ketamine/xylazine protocol upon antipyrine clearance is disturbing in that this regimen is quite often adopted, but no previous observation of an inhibitory effect of ketamine upon microsomal metabolism has been made. Of relevance to this present observation may be the report by Waterman (24), demonstrating that a 1-mg/kg injection of xylazine in the cat can significantly inhibit hepatic microsomal oxidation, with an almost twofold prolongation in the terminal half-life of coadministered ketamine.

To conclude, this report provides an initial observation of the differential influence that some laboratory anesthetic protocols may have upon hepatic metabolism. Further work is required to investigate these observations. The use of the conscious chronically catheterized animal should be considered preferable in laboratory pharmacokinetic studies.

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