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The Extent of Postmortem Drug Redistribution in a Rat Model

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ABSTRACT: The aim of this study was to investigate the postmortem redistribution of several drugs in a rat model and to examine if any of the pharmacological properties was related to the extent of this phenomenon. One of the following drugs: phenobarbital (phenobarbitone), acetaminophen (paracetamol), carbamazepine, codeine, verapamil, amphetamine, mianserin, trimeprazine (alimemazine) or chloroquine was administered together with nortriptyline orally to rats 90 min prior to sacrifice. Heart blood was sampled immediately before sacrifice and after 2 h postmortem, as it has previously been shown that this is sufficient time for postmortem concentration changes to occur in heart blood. Blood was also sampled from the clamped abdominal inferior vena cava (representing peripheral blood) and tissue samples were taken from lungs, myocardium, liver, kidney, thigh muscle, forebrain, and vitreous humor together with a specimen from the minced carcass. Drugs were analyzed by high performance liquid or gas chromatography. For phenobarbital, acetaminophen and carbamazepine the postmortem to antemortem blood drug concentration ratios were close to 1.0 and tissue concentrations were low. The postmortem to antemortem heart blood drug concentration ratio for chloroquine (6.9 ± 1.5) was higher than for nortriptyline (3.5 ± 0.3), and the remaining drugs (codeine, verapamil, amphetamine, mianserin, and trimeprazine) showed ratios of the same magnitude as nortriptyline. The postmortem to antemortem blood drug concentration ratios for both heart blood and blood from the vena cava and also the lung to antemortem blood drug concentration ratio were closely related to the apparent volume of distribution for the drugs studied ($p < 0.001$). Accordingly, an apparent volume of distribution of more than 3–4 L/kg is a good predictor that a drug is liable to undergo postmortem redistribution with significant increments in blood levels. The postmortem drug concentration in blood from vena cava was closely related to the antemortem blood level, confirming that among the postmortem samples, the peripheral blood sample was the most representative for the antemortem blood concentration.

KEYWORDS: forensic science, forensic toxicology, postmortem redistribution, pharmacokinetics, rats, tissue distribution, nortriptyline, phenobarbital (phenobarbitone), acetaminophen (paracetamol), carbamazepine, codeine, verapamil, amphetamine, mianserin, trimeprazine (alimemazine), chloroquine

The phenomenon of postmortem drug redistribution makes interpretation of drug concentrations found in postmortem blood samples difficult. Ignorance of this phenomenon may well lead to erroneous interpretations. Postmortem redistribution has been reported to occur for many different drugs in both humans and experimental animal models. Drugs like tricyclic antidepressants, barbiturates, opiates, and others have been reported to show postmortem concentration increases in humans (1,2). On the other hand there are reports on substances that redistribute insignificantly postmortem, such as acetaminophen (paracetamol) and zopiclone (3,4). Several experimental animal models have been employed to shed light on this phenomenon and rats have been used to investigate postmortem morphine (5,6), amitriptyline (7–9), triazolam (10), and secobarbital (11) concentration changes.

After death several mechanisms can give rise to artificially increased blood drug concentrations. Firstly, a drug may be released postmortem from tissues with high drug concentrations and redistribute by means of diffusion and convection of blood and other fluids in the body. The lungs have been experimentally verified as a source of postmortem drug release to the blood (9), but also liver, myocardium, endothelium, and kidneys have been suggested as possible sources. Secondly, drug diffusion from unabsorbed gastrointestinal drug depots to anatomically adjacent tissues like the left lobe of the liver, left lower lobe of the lung and eventually myocardium and blood in central compartments has been reported (8,12,13). Thirdly, agonal or postmortem reflux of drug-rich material from the stomach into airways followed by release to the blood can give rise to falsely elevated drug concentrations in heart blood and other central tissues (8,14).

In this study we have administered different drugs orally to rats and compared the changes found in each animal to nortriptyline, a substance with well documented postmortem redistribution potential (7,15–17). By giving nortriptyline as a reference substance it was possible to determine how the drugs tested compared with this drug in spite of large individual differences. A protocol comparable to a previous study (7) was applied, the main differences being that 30 mg nortriptyline was used instead of 75 mg amitriptyline and that a shorter interval between drug administration and sacrifice ($1^1/_2$ h vs. 2 h) was used. Nortriptyline was chosen over amitriptyline due to lack of chromatographic interference with the other substances tested and less toxicity (18). The antemortem interval was reduced to compensate for the short half-life of these drugs in rats (19,20).

The aims of this study were to investigate to what extent the different drugs redistribute to heart blood and to blood in the vena cava postmortem and how they distribute in the different tissues relative to nortriptyline. We also wished to explore if the increase seen in the drug concentration in heart blood and blood from the vena cava was related to either the pharmacological properties of that particular drug or to the tissue to blood drug concentration ratios for the different parenchymal tissues.

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Materials and Methods

Chemicals and Drugs

Potassium fluoride solution was made of 67% (w/v) KF in water. Acetonitrile was of HPLC grade (Fisons, UK), and all the other chemicals were of analytical grade. Phenobarbital, acetaminophen, codeine phosphate, verapamil hydrochloride, amphetamine sulfate, and chloroquine diphosphate were all obtained from commercial sources. Nortriptyline hydrochloride was obtained from Sigma (USA), trimeprazine tartrate from Rhône-Poulenc Rorer (France), carbamazepine from Ciba-Geigy (Switzerland), and mianserin hydrochloride from Organon (The Netherlands).

Animal Studies

Male Wistar rats with a body weight of 230–310 g were fasted overnight and administered 30 mg of nortriptyline hydrochloride in addition to one of the drugs listed above in 2 mL of water by a gastric tube. The dosages are outlined in Table 1. The rats were anaesthetized 90 min after dosing with a subcutaneous injection of a mixture of fluanisone, fentanyl, and midazolam (5 mg, 0.1 mg, and 2.5 mg per kg, respectively). A heart blood sample (≈600 μL) was drawn and divided into 3 aliquots in Eppendorf tubes containing 25 μL KF solution. The rats were then sacrificed with CO_2 and left at room temperature for 2 h prior to autopsy. Postmortem heart blood samples (≈600 μL divided into 3 aliquots) were drawn after clamping the inferior vena cava just above the diaphragm. Blood from the inferior vena cava (≈400 μL) was sampled after clamping proximal to the renal veins. The following tissue samples were also collected: vitreous humor from both eyes (≈0.12 g), tissue from the upper lobe of the right and left lungs, the heart, one sample from the right part of the median liver lobe and another from the left caudate liver lobe lying next to the stomach, lower pole of right kidney, thigh muscle and forebrain (≈0.5 g each). In addition the stomach was dissected out (≈6.6 g) and the proximal 50 cm of the small intestine (≈4.0 g) was sampled. The carcasses were minced in a manual meat mincer twice and 5 g were obtained for further homogenization like the other tissues. The total amount of drug in the body was calculated as the product of drug concentration in this homogenate and initial body weight, plus the amount of drug in stomach and small intestine.

Extraction, Chromatography, and Analytical Procedure

Tissue samples were homogenized with an Ultra-Turrax T5 homogenizer (IKA, Janke & Kunkel, Germany) in water to a final concentration of 0.2 g tissue/mL homogenate, except the stomach and intestine that were diluted to 0.002 and 0.02 g tissue/mL, respectively. Drug concentrations in blood and vitreous humor samples were determined from a calibration curve prepared from blood, while tissue concentrations were determined from a calibration curve prepared from drug-spiked liver tissue samples.

Samples containing carbamazepine, acetaminophen or phenobarbital were analyzed by adding 100 μL of an aqueous solution of internal standard to tissue and blood samples. As internal standards we used methaqualone for carbamazepine analyses, 3-acetoaminophenol for acetaminophen and cyclobarbital for phenobarbital assays. The mixture was extracted in a mixer with 800 μL of ethylacetate for 10 min, and centrifuged for 10 min at 740 g. The organic phase was transferred to 1.1 mL autosampler vials, evaporated at 50°C under vacuum (Buchler Vortex Evaporator, USA) and the residue was reconstituted with 100 μL of the mobile phase. The liquid chromatograph consisted of a SCL 6A system controller, LC 6A pump, and SIL 6A autosampler (Shimadzu Corp, Japan) and a Perkin Elmer LC-235 diode array detector (USA). Chromatography was performed at ambient temperature on a Hypersil 5 μm, ODS 200 × 4.6 mm column (HP 79916 OD-574) (Hewlett Packard, USA). The mobile phase for carbamazepine consisted of 0.002 M phosphoric acid/0.02% triethylamine: acetonitrile (70:30), for acetaminophen acetic acid 1%: acetonitrile (80:20) and for phenobarbital 0.05 M Na_2HPO_4/0.05 M NaH_2PO_4/methanol/acetonitrile (32.5: 31.5: 31:5), with flow rates of 2.5, 1.7, and 1.5 mL/min, respectively. The injection volume was 20 μL. The UV-detector was set at 285, 245, and 230 nm for carbamazepine, acetaminophen, and phenobarbital, respectively. Calibration curves were prepared for each analysis and were determined from four standards analyzed in duplicate at the beginning and end of each series. Recovery from blood exceeded 72% for carbamazepine and phenobarbital and 48% for acetaminophen at

TABLE 1—*The dosage, the antemortem heart blood drug concentration (μmol/L), the postmortem to antemortem heart blood drug concentration ratio and the postmortem vena cava to antemortem heart blood drug concentration ratio (mean ± standard error of the mean) in rats sacrificed at 90 min. Autopsy and sampling was performed 2 h postmortem. Four animals were excluded due to possible airways contamination. The data for the reference substance nortriptyline are pooled for all animals.*

	n	Dosage (mg)	Antemortem Heart Blood Drug Concentration (μmol/L)	Postmortem to Antemortem Heart Blood Drug Concentration Ratio	Postmortem vena cava to Antemortem Heart Blood Drug Concentration Ratio
Phenobarbital	5	100	180 ± 38	0.9 ± 0.1*	0.9 ± 0.0*
Acetaminophen	5	100	229 ± 37	1.0 ± 0.0*	1.1 ± 0.0*
Carbamazepine	6	100	24 ± 5	1.1 ± 0.1*	1.5 ± 0.5
Codeine	6	30	3.3 ± 0.3	2.2 ± 0.3	2.3 ± 0.8
Mianserin	6	30	1.7 ± 0.2	2.3 ± 0.2	1.6 ± 0.3
Amphetamine	6	30	9.4 ± 3.5	2.4 ± 0.2	2.3 ± 0.3
Verapamil	5	30	1.6 ± 0.6	2.7 ± 0.4	2.0 ± 0.3
Trimeprazine	5	30	0.4 ± 0.1	6.3 ± 3.0	2.4 ± 0.4
Chloroquine	6	30	2.2 ± 0.2	6.9 ± 1.5†	3.4 ± 0.4†
Nortriptyline	50	30	0.5 ± 0.1	3.5 ± 0.3	1.9 ± 0.2

* Significantly lower than the reference substance nortriptyline ($p < 0.05$).
† Significantly higher than the reference substance nortriptyline ($p < 0.05$).

concentrations of 10 and 300 μmol/L. Recovery from tissues exceeded 79% for carbamazepine and phenobarbital and 59% for acetaminophen at concentrations of 10 and 300 μmol/L. The calibration ranges were from 5 to 300 μmol/L in blood and from 1.8 to 300 μmol/kg in tissue and there was linearity within the entire range ($R^2 > 0.99$). The coefficient of variation (CV) of within-run precision for acetaminophen, carbamazepine and phenobarbital was better than 8% in blood and 9% in liver tissue at concentrations of 10 and 300 μmol/L.

Nortriptyline, verapamil, mianserin, codeine, and trimeprazine were analyzed by high performance liquid chromatography according to a slight modification of a previously reported method (21). In the present study extraction was performed using 0.8 mL ethylacetate/heptane (4:1) and separation was achieved by a 250 × 4.6 mm 5 μm LC-CN column (Supelco, USA). The mobile phase consisted of 10% v/v 0.05 M ammonium acetate in acetonitrile and the ultraviolet detector was set at 254 nm. Recovery from blood and tissue exceeded 80% for these substances at concentrations of 1 and 10 μmol/L. The calibration ranges were from 0.24 to 30 μmol/L in blood and from 1.2 to 150 μmol/kg in tissue and there was linearity within these ranges ($R^2 > 0.99$). The coefficient of variation (CV) of within-run precision for these substances was better than 9% at concentrations of 1 and 10 μmol/L.

The samples with chloroquine were kept in iced silanized glass tubes to which 100 μL of an aqueous solution of the internal standard promazin and 300 μL 1 M Tris (pH = 10.7) were added. The mixture was extracted for 10 min with 400 μL of butylacetate and centrifuged for 10 min at 740 g. The organic phase was transferred to 100 μL autosampler vials. The gas chromatograph was a HP 5890, and the column was a polar 15 m × 0.32 mm DB 1701, 0.32 μm film thickness (J&W Scientific, USA). The injection volume was 5 μL (split mode 1:10) and a nitrogen-phosphor detector was employed. The injector temperature was 250°C and the detector temperature was 300°C. The temperature program started at 150°C for 1 min and increased by 20°C per min to 250°C with a hold time of 0.5 min, with further increments of 5°C per min to 280°C with a hold time of 10 min. The carrier gas was helium with a flow rate of 1.5 mL/min and make up gas to a total of 30 mL/min. Recovery in blood and liver tissue exceeded 85% for chloroquine at concentrations of 1 and 10 μmol/L. The calibration ranges were from 0.25 to 20 μmol/L in blood and 1.8 to 300 μmol/kg in tissue and there was linearity within these ranges ($R^2 > 0.99$). The coefficient of variation (CV) of within-run precision was better than 5% in both blood and tissue at concentrations of 1 and 10 μmol/L.

Blood samples containing amphetamine were extracted with cyclohexane and the extracts were derivatized with perfluorooctanoyl chloride prior to gas chromatography/mass spectrometry according to a previously reported method (22). The analytical parameters for rat blood and liver were found to be comparable to those for human blood.

Values for apparent volume of distribution for rats were obtained from the literature for phenobarbital (23), acetaminophen (24), carbamazepine (25), codeine (26), verapamil (27), amphetamine (28), mianserin (29), and nortriptyline (20). For chloroquine the mean human value was applied (30), while for trimeprazine no reference values were found and the value calculated from the present study was applied. The apparent volume of distribution is defined as the amount of drug in body divided by plasma or blood drug concentration and body weight when distribution equilibrium has been attained (31). Accordingly, the apparent volume of distribution in the present study was calculated by dividing the drug concentration in carcass homogenate by the drug concentration in antemortem heart blood.

Results are presented as mean ± standard error of the mean (s.e.m.) in μmol/L for blood and μmol/kg for tissues and statistical analysis was performed using Minitab ver. 10.5 (Minitab Inc., PA, USA). The non-parametric Wilcoxon or Mann-Whitney tests were applied for verifying differences between groups, while regression analysis was used for detecting covariation and results with $p < 0.05$ were considered significant. The results for nortriptyline were assessed separately from the other drugs.

Results

The blood concentrations found in antemortem heart blood were generally in a range associated with therapeutic to toxic effects in humans (Table 1). Correspondingly, the rats that received amphetamine became restless and showed stereotyped behavior with repetitive movements and hyperactivity, while the rest of the animals became sedated. In four animals very high postmortem blood and lung concentrations were found (more than 5 times higher than the rest). This was interpreted as being due to agonal or postmortem esophageal reflux of drug-rich stomach contents to the lungs and the animals were accordingly excluded from further evaluation (8,14).

The postmortem to antemortem heart blood drug concentration ratios for the animals that received the acidic/neutral drugs phenobarbital, acetaminophen or carbamazepine were close to 1.0, which was significantly lower than for nortriptyline and the variability was small (Table 1). For the basic drugs codeine, mianserin, amphetamine, verapamil, and trimeprazine there were significantly elevated postmortem to antemortem heart blood drug concentration ratios with relatively large variability and the changes observed were not significantly different from nortriptyline. Only for chloroquine was the increase observed significantly higher than for nortriptyline ($p < 0.05$). The postmortem vena cava to antemortem heart blood drug concentration ratios followed a similar pattern, but the changes were generally less pronounced (Table 1). For phenobarbital and acetaminophen the changes were significantly smaller than for nortriptyline and for chloroquine the increase was significantly higher than for nortriptyline ($p < 0.05$). The postmortem heart to vena cava blood drug concentration ratios were significantly elevated only for nortriptyline (1.9 ± 0.2, $p < 0.05$) (data not shown).

The postmortem tissue to antemortem blood concentration ratios are outlined in Table 2. The ratios for the acidic/neutral drugs phenobarbital, acetaminophen, and carbamazepine were well below 1.0 in carcass homogenate as well as in all tissues analyzed and there was little variability. For the other drugs and the reference substance nortriptyline the following significant differences were noted between the tissue to antemortem blood drug concentration ratios: there was a general and marked increase of this ratio for nortriptyline in all tissues studied, ranging from 2 (vitreous humor) to more than 70 (liver and lung). The other basic drugs showed similar changes with the exception of amphetamine, where the carcass homogenate to antemortem blood concentration ratios were lower than for codeine and verapamil, which again were lower than for nortriptyline. The lung to antemortem blood concentration ratios for amphetamine, codeine, and verapamil as well as for mianserin and trimeprazine were lower than for nortriptyline, whereas chloroquine distributed similarly to nortriptyline. The myocardium to antemortem blood concentration ratios for amphetamine were significantly lower than for nortriptyline and the liver to antemortem

TABLE 2—*The postmortem tissue to antemortem blood drug concentration ratio (mean ± standard error of the mean) in rats administered the drugs and sacrificed after 90 min. The data for the reference substance nortriptyline are pooled for all animals.*

	Carcass Homogenate	Lung	Myocardium	Liver	Kidney	Muscle	Brain	Vitreous Humor
Phenobarbital	0.10 ± 0.01*	0.09 ± 0.01*	0.13 ± 0.01*	0.20 ± 0.01*	0.16 ± 0.01*	0.05 ± 0.01*	0.07 ± 0.01*	0.32 ± 0.03*
Acetaminophen	0.05 ± 0.01*	0.15 ± 0.01*	0.09 ± 0.01*	0.19 ± 0.01*	0.15 ± 0.02*	0.10 ± 0.01*	0.14 ± 0.01*	0.34 ± 0.05*
Carbamazepine	0.08 ± 0.01*	0.3 ± 0.1*	0.29 ± 0.07*	0.32 ± 0.08*	0.75 ± 0.20*	0.47 ± 0.08*	0.24 ± 0.07*	0.32 ± 0.14*
Codeine	6.6 ± 1.1*	6.1 ± 2.4*	2.5 ± 0.3	21.9 ± 1.7*	12.4 ± 3.0*	2.1 ± 0.4	2.1 ± 0.3	0.9 ± 0.3
Mianserin	18.1 ± 6.1	23.3 ± 2.4*	3.1 ± 0.9	58 ± 10	16.2 ± 1.0*	2.2 ± 0.3	13.7 ± 1.4	1.1 ± 0.2
Amphetamine	1.0 ± 0.2*	1.8 ± 0.1*	0.9 ± 0.1*	2.0 ± 0.7*	6.3 ± 2.0*	0.8 ± 0.2	1.8 ± 0.6*	1.1 ± 0.2
Verapamil	7.4 ± 1.7*	25.5 ± 2.6*	5.9 ± 0.5	45 ± 7*	27.5 ± 10.3*	3.8 ± 1.1	1.9 ± 0.3*	0.8 ± 0.2
Trimeprazine	51 ± 10	31 ± 13*	8.2 ± 2.1	106 ± 35	44 ± 24	1.4 ± 0.4	10.8 ± 4.1	0.8 ± 0.4
Chloroquine	6.4 ± 1.5	43 ± 10*	2.9 ± 0.4	55 ± 8	23.5 ± 1.9	1.6 ± 0.1	0.7 ± 0.3*	0.8 ± 0.1
Nortriptyline	27.4 ± 5.5	71 ± 6	7.0 ± 0.7	72 ± 9	34 ± 4	3.2 ± 0.4	10.5 ± 1.1	2.3 ± 0.9

* Significantly lower than the reference substance nortriptyline ($p < 0.05$).

TABLE 3—*The mean apparent volume of distribution (Vd) in man (L/kg) (30,32), Vd in rats (20,23–29) and calculated apparent volume of distribution at time of death in the present study (L/kg) (mean ± standard error of the mean). The data for the reference substance nortriptyline are pooled for all animals.*

	Mean Apparent Volume of Distribution (Vd) in Man (L/kg)	Apparent Volume of Distribution in Rats (L/kg)	Calculated Vd in the Present Study (L/kg)
Phenobarbital	0.7	0.6	0.10 ± 0.01
Acetaminophen	0.9	1.0	0.05 ± 0.01
Carbamazepine	1.4	1.1	0.08 ± 0.01
Codeine	3.6	5.1	6.6 ± 1.1
Mianserin	13	8.6	18.1 ± 6.1
Amphetamine	4.2	4.1	1.0 ± 0.2
Verapamil	5.5	5.1	7.4 ± 1.7
Trimeprazine	—	—	51 ± 10
Chloroquine	200	—	6.4 ± 1.5
Nortriptyline	27	13.7	27 ± 6

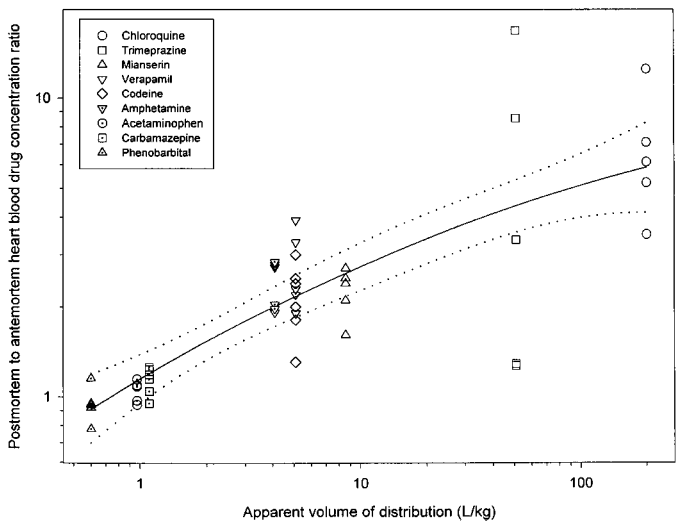

FIG. 1—*The apparent volume of distribution in rats versus the postmortem to antemortem heart blood drug concentration ratio and regression line with 95% confidence interval ($R^2 = 0.65$, $p < 0.001$). Please note logarithmic scales.*

blood concentration ratios for codeine, amphetamine, and verapamil were significantly lower than the corresponding ratios for nortriptyline. The kidney to antemortem blood concentration ratios for codeine, mianserin, amphetamine, and verapamil were significantly lower than for nortriptyline. There were no significant differences in the muscle or vitreous humor to antemortem blood drug ratios for any of the basic drugs. The brain to antemortem blood concentration ratios for amphetamine, verapamil, and chloroquine were lower than the corresponding ratios for nortriptyline. There was a tendency towards higher drug concentration in the left caudate lobe of the liver than in the right part of the median lobe, but statistical significance was found only for nortriptyline with a left to right lobe concentration ratio of 2.8 ± 0.6.

The mean apparent volumes of distribution in man (30,32) and rats (20,23–29) obtained from the literature were reasonably comparable (Table 3). In the present study the apparent volumes of distribution calculated for the acidic/neutral drugs appears low. For the basic drugs, however, the results were of the same order of magnitude as those obtained from the literature. There was correlation between the postmortem to antemortem heart blood drug concentration ratios and the apparent volume of distribution for the different drugs (Table 4, Fig. 1). The postmortem to antemortem heart blood drug concentration ratios also correlated with the lung, myocardium, liver, and carcass homogenate to antemortem blood drug concentration ratio (Table 4), as shown for the lungs in Fig. 2. The postmortem vena cava to antemortem heart blood drug concentration ratios showed covariation with both the apparent volume of distribution for the different drugs, the lung, myocardium, and liver to antemortem blood drug concentration ratios. The drug concentration in the blood from the vena cava showed the best correlation with the antemortem blood drug concentration, and this also applied for nortriptyline ($p < 0.001$). The postmortem drug concentration in vitreous humor as well as myocardium was closely related to the antemortem blood concentration of the different drugs ($p < 0.001$). The drug concentration in the carcasses was related to the apparent volume of distribution and the drug concentration in the myocardium ($p < 0.005$ and $p < 0.05$, respectively), but was unrelated to the drug levels in any of the other tissues. The concentration of nortriptyline in the carcasses was related to the drug concentration in the kidney and postmortem heart blood ($p < 0.001$ and $p < 0.01$, respectively).

The apparent volume of distribution was significantly correlated to the tissue to antemortem blood concentration ratios for liver

TABLE 4—*Correlation between the postmortem to antemortem heart blood drug concentration ratios and the postmortem vena cava to antemortem heart blood drug concentration ratios versus the apparent volume of distribution and the tissue to antemortem blood drug concentration ratios using regression analysis. The results of the reference substance nortriptyline are not included (n.s. = not significant).*

	Postmortem to Antemortem Heart Blood Drug Concentration Ratio		Postmortem Vena Cava to Antemortem Heart Blood Drug Concentration Ratio	
	R^2	p	R^2	p
Volume of distribution (L/kg)	0.65	< 0.001	0.33	< 0.001
Lung to antemortem heart blood drug concentration	0.54	< 0.001	0.27	< 0.001
Myocardium to antemortem heart blood drug concentration	0.34	< 0.001	0.09	< 0.05
Liver to antemortem heart blood drug concentration	0.50	< 0.001	0.10	< 0.05
Kidney to antemortem heart blood drug concentration		n.s.		n.s.
Muscle to antemortem heart blood drug concentration		n.s.		n.s.
Carcass to antemortem heart blood drug concentration	0.15	< 0.01		n.s.

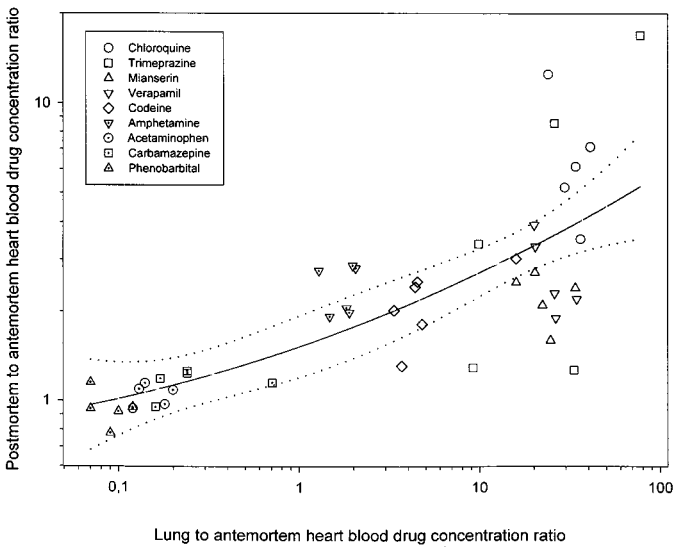

FIG. 2—*The lung to antemortem heart blood drug concentration ratio versus the postmortem to antemortem heart blood drug concentration ratio and regression line with 95% confidence interval (*$R^2 = 0.54$, $p < 0.001$*). Please note logarithmic scales.*

($R^2 = 0.48$, $p < 0.001$), kidney ($R^2 = 0.39$, $p < 0.001$), lung ($R^2 = 0.34$, $p < 0.001$), myocardium ($R^2 = 0.24$, $p < 0.001$), carcass ($R^2 = 0.15$, $p < 0.01$) and muscle ($R^2 = 0.14$, $p < 0.05$). There did not seem to be any relationship between the postmortem concentration changes in the present study and pharmacological parameters such as the acid dissociation constant (pKa), molecular size, plasma protein binding or lipophilicity (data not shown).

For phenobarbital, acetaminophen, carbamazepine, and amphetamine a relatively high fraction of the administered dosage was found in the blood (≈0.1–1%), while for the other basic drugs a smaller fraction was retrieved (≈0.01–0.1%) (data not shown). Only about 2–4% of the dosage of the acidic/neutral drugs was recovered in the body. At autopsy the stomachs were distended and had a mean fluid content of 4.4 ± 0.2 g, more than twice the administered volume. For the acidic/neutral drugs only about 1.5–3% of the administered dose was recovered from the stomach, while for the basic drugs a much larger fraction was retrieved (≈16–60%) (data not shown).

Discussion

The concentration changes observed for nortriptyline in heart blood and blood from the vena cava at 2 h postmortem in the present study were in accordance with a previous study (7), and are comparable with changes seen in larger experimental animals and humans after a few days (1,16,21). Diffusion from the unabsorbed drug depot in the stomach at 2 h postmortem is limited mainly to the left lobe of the liver (8), and could not explain the postmortem drug concentration increases. A protracted postmortem interval in small animals like rats may cause a vast increase in the drug concentration in both the abdominal cavity and thorax due to drug diffusion from gastrointestinal depots. Several previous reports have not taken this mechanism into account and this has given rise to misinterpretations (33,34). Diffusion from gastrointestinal drug depots has also been demonstrated in humans (12,35). Agonal or postmortem reflux can be avoided under experimental conditions by ligation of the esophagus and/or trachea (8), but in order to minimize postmortem body handling, this was not performed in the present study.

Due to the large variability in postmortem redistribution, nortriptyline was administered to all animals as a reference substance to facilitate the detection of minor differences between the drugs. Nortriptyline can be utilized as a "biological internal standard" by dividing the postmortem to antemortem drug concentration ratio for the different drugs with the corresponding result for nortriptyline in each animal. In this way less inter-individual variability for the basic lipophilic drugs were obtained, but the variability for the acidic/neutral drugs increased correspondingly, giving no net advantage ($R^2 = 0.67$, $p < 0.001$). However, the correlation between postmortem drug concentration increase and apparent volume of distribution was confirmed. In the present study phenobarbital, acetaminophen, and carbamazepine demonstrated less postmortem redistribution to heart blood than nortriptyline, while chloroquine had more. By administering more than one drug to all animals, the possibility of interactions arise. The large amount of the basic lipophilic drugs found in the stomach in the present study was probably due to the anticholinergic effects of nortriptyline and to ion trapping in this acidic medium (19), an effect also observed in humans (36). The gastric retention seemed to be enhanced by trimeprazine and codeine, while amphetamine appeared to counteract this effect, as there was little nortriptyline left in the stomach in these rats (data not shown). Drug distribution in tissues may also have been affected. A previous report showed that basic lipophilic drugs may interact through inhibition of accumulation in the iso-

lated perfused lung depending on their lipid solubility (37). Recently it has been shown that the lungs function as a reservoir for antidepressants (38). Addition of a second antidepressant caused release of the first drug in volunteers, with an increased risk of toxicity. It has been established that cationic amphiphilic drugs accumulate in tissues by two major mechanisms, namely non-specific binding to membrane phospholipids and ion trapping within acidic cellular compartments like lysosomes (39). Tissues like lung, brain, heart, and kidney were found to accumulate desipramine and chloroquine in a tissue-specific and strongly drug-dependent manner.

The tissue to blood drug concentration ratio of the lungs was found to be predictive of the postmortem drug level increase observed in heart blood. This is in agreement with a previous study in rats showing that removal of the lungs significantly reduced the postmortem drug level increase in heart blood for amitriptyline (9). The apparent volume of distribution is defined as the amount of drug in the body divided by plasma or blood concentration at distribution equilibrium. The ratio of the drug concentration in the carcass to the drug concentration in antemortem blood accordingly expresses the apparent volume of distribution at time of sampling/death. We found agreement between the apparent volume of distribution calculated in the present study and previous findings only for verapamil and codeine. However, distribution equilibrium was almost certainly not achieved for all of the drugs tested. The reason for the low values obtained for the acidic/neutral drugs is not known. It may partly have analytical reasons, as the recovery for these drugs was low, but the use of internal standard should compensate for this. Also, interactions from nortriptyline can influence these results. Nevertheless, the highly significant covariation between the postmortem drug concentration increase and the apparent volume of distribution shows that the latter is a very useful measure for assessing drugs for the possibility of postmortem redistribution. The very close relationship between the tissue to antemortem blood concentration ratios and the apparent volume of distribution found in the present study corroborates the fact that the apparent volume of distribution of a drug also expresses the average tissue to blood drug concentration ratio. Furthermore, it is a measure for the concentration gradient between tissue and blood. Fick's law of diffusion states that the rate of diffusion of a substance is proportional to the concentration gradient across the diffusion barrier. Consequently, the apparent volume of distribution is a logical measure for the liability of a drug to redistribute from tissues to blood after death.

The results for acetaminophen, phenobarbital, and carbamazepine were in most respects different from the other drugs. These drugs are acidic or neutral and have apparent volumes of distribution of around 1 L/kg in both man and the rat (Table 2) and consequently the tissue concentrations were low (Table 3). The non-ionic properties of these moieties may explain why they were preferentially absorbed from the acidic stomach contents, while nortriptyline and the other basic lipophilic drugs were poorly absorbed. There were remarkably little concentration changes postmortem, as confirmed by others (3,40). This may also support the assumption that the postmortem concentration changes seen in the present experimental model were due to the same mechanisms as those seen in man, without undue interference from other mechanisms such as direct diffusion from gastrointestinal depots. Recently large postmortem acetaminophen level increases were found in a rabbit model (33). The discrepancy between this study and the present results may well be due to direct diffusion of drug from gastrointestinal depots to blood, alternatively to agonal or postmortem esophageal reflux to the airways.

Chloroquine had consistently higher postmortem to antemortem heart blood concentration ratios than nortriptyline. Previously tissue to plasma concentration ratios of 200–500 have been reported after chronic administration to rats (41,42), but distribution equilibrium was still not achieved after 7 days. Chloroquine differs from the other basic lipophilic drugs due to its very high apparent volume of distribution of ≈200 L/kg (30) and a very long half-life of 20–60 days in man. The drug has been found to be trapped within lysosomes and has been detected in urine specimens up to one year after the last dose (42). Accordingly, more pronounced postmortem redistribution would be expected after chronic treatment with this drug and a report on human cases recommended liver tissue as the best specimen for interpretation of postmortem cases (43). The lower tissue to blood drug concentration ratios and apparent volume of distribution found in the present study are probably due to the fact that distribution equilibrium was far from established at time of death.

The heart to femoral blood drug concentration ratio has been extensively studied in human case material and if high this indicates that the drug is subject to postmortem redistribution (1,16). The present study corroborates these reports (Table 1) and shows that in rats as well, peripheral blood specimens are more representative for the antemortem situation than blood from heart or central vessels (44). The muscle to antemortem blood drug concentration ratios in the present study were generally higher than those reported in man (45), also when compensating for the use of antemortem blood. Muscle tissue constitutes about 30% of the body weight in rats (46) and drug quantification in this tissue may prove valuable for interpretation of postmortem cases as a relatively large proportion of total body drug is found in this tissue (Table 4).

The low fraction of administered dose found in tissues and carcass in the present study indicates that the drugs studied are eliminated efficiently in rats. The oral bioavailability in rats of codeine is reported as 8 ± 3% (26), verapamil as 21% (47), and amitriptyline as 4–5% (20), indicating extensive first pass metabolism. The half-life of most drugs is also much shorter than in man (19).

We conclude that this experimental animal model may offer a strategy towards predicting if a drug is subject to significant postmortem drug redistribution from tissues of high concentration to blood, comparable to previous findings in humans. An apparent volume of distribution of more than 3 to 4 L/kg indicates that a drug is liable to postmortem drug redistribution, and if so, the extent of this phenomenon is variable. Some previous reports have probably misinterpreted postmortem increases in blood concentration as due to redistribution from tissues, while gastro-intestinal sources are more likely. In order to increase diagnostic accuracy determination of tissue drug concentrations may be rewarding, but reference data will have to be established.

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