Pharmacokinetics of antipyrine, paracetamol, and morphine in rat at 71 ATA

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Aanderud L, Bakke OM. Pharmacokinetics of antipyrine, paracetamol, and morphine in rat at 71 ATA. Undersea Biomed Res 1983; 10(3):193–201. — The pharmacokinetics of [14C]antipyrine, [3H]paracetamol, and [3H]morphine were studied in male Wistar rats at 1 ATA and then at 71 ATA, each animal being its own control. Separate control groups were studied at 1 ATA in order to test the validity of the one-way crossover protocol. The drugs were injected through a femoral venous catheter, and blood was sampled from a catheter in the femoral artery. There were no significant differences in the biological half-life (\(T_{1/2}\)), the apparent volume of distribution (\(V_d\)), and the clearance (\(C\)) of antipyrine and paracetamol. Likewise, there was no appreciable effect of high pressure on the blood concentration profile of morphine.

Several reports have appeared during the last decade indicating that the effect of barbiturates (1), ketamine (1), diazepam (2), alphaxalone/alphadalone (3), and isoflurane (4) is reduced in mammals exposed to high pressure. The same phenomenon has been demonstrated in human volunteers receiving alphaxalone/alphadalone at 31 ATA (5, 6). Some central nervous stimulants and depressants have unpredictable effects even at moderately raised pressures (7). These observations may be ascribed to pressure-induced changes in function of excitable cell membranes (8).

High pressure also affects the properties of nonexcitable membranes (8, 9) and could conceivably interact with the distribution, metabolism, and excretion of drugs. A few in vitro studies on drug metabolism at high pressure have been published (10–13), but in vivo data are available only for meperidine at 6 ATA (14) and for morphine at 11 ATA and 21 ATA (15).

The present report deals with the in vivo elimination in the rat at 71 ATA of three common drugs with different metabolic pathways. The drugs chosen for the experiments were antipyrine, which is metabolized by oxidative processes (16) and has been extensively studied; paracetamol, which is conjugated with sulfate and glucuronic acid (17); and morphine, which is eliminated by conjugation with glucuronic acid and demethylation (18) during the passage through the liver (19).
MATERIALS AND METHODS

Animal preparation

Male Wistar rats weighing 200–300 g were used. They were prepared as described (20) with a PE-50 polyethylene catheter in the femoral artery and another in the femoral vein, inserted during fluanisone/fentanyl anesthesia (20). The catheters were led subcutaneously to the flank. The animals were kept in restraining tubes during the experiments and had free access to pellet diet. Adequate hydration and anticoagulation were ensured by the intravenous infusion of 5 ml isotonic saline and 200 IU of heparin each day of the experiment.

Compression procedure

A 4-liter pressure chamber designed for pharmacological experiments in small animals was used (20). The chamber was pressurized to 4 ATA with air and from then on with helium, proceeding at a rate of 1 ATA/min. After reaching 71 ATA, 1 h was spent adapting to the pressure before the experiments started. The partial pressure of oxygen was monitored with a paramagnetic oxygen analyzer (Servomex DA 580, Taylor & Servomex Ltd., Crowborough, Sussex, U.K.). Oxygen was added as necessary to keep its partial pressure at 0.4 ATA. The rectal and atmospheric temperatures were measured by two integrated circuit temperature transducers (AD 590, Analog Devices Inc., Norwood, MA.). Gas mixing was achieved by a fan driven by an induction motor. Moistened soda lime granules were spread out in a tray on the chamber floor in order to keep the CO₂-concentrations below 0.01% v/v (measured by CO₂ test tubes, Drägerwerke, Lübeck, W. Germany). The animals were killed by rapid decompression in the pressure experiments, whereas the control group received an anesthetic overdose.

Drugs

[N-methyl-³¹C] antipyrine was obtained from New England Nuclear (Boston, MA) and diluted with the unlabeled drug (BDH, Poole, Dorset, U.K.) in 0.9% sodium chloride. This solution (5 mg/ml, 0.5–2.0 µCi/mg), corresponding to a dose of 15 mg/kg, was injected intravenously. The injection time was 60 s.

Generally labeled ³¹H]paracetamol (New England Nuclear) was diluted with the unlabeled drug (NAF, Oslo, Norway) and dissolved in 0.9% NaCl to a final solution containing 10 mg/ml, 1.7–3.4 µCi/mg. A dose of 15 mg/kg was injected intravenously.

[1-³¹H]morphine was obtained from Amersham Int., Ltd. (Amersham, Bucks, U.K.) and diluted with the unlabeled drug (NAF, Oslo, Norway) in 0.9% NaCl. This solution (1 mg/ml, 4.8–7.1 µCi/mg), corresponding to a dose of 2.5 mg/kg, was injected intravenously.

Antipyrine experiments

Five rats were studied on two consecutive days, first at 1 ATA and the next day at 71 ATA. A control group of 5 animals kept at 1 ATA was included in order to test the protocol, particularly with regard to the effect of anesthesia, surgery, and restraint. The first injection was given via the femoral vein catheter 3 h after the anesthetic dose for the catheter insertion had been given intraperitoneally. Whole-blood samples (100 µl) were collected from the arterial catheter into capillary glass tubes. A second dose was given the next day at 71 ATA. The control group was injected at 1 ATA air on two consecutive days. The blood samples from the
pressure experiments were collected into syringes, which were weighed with their contents and then reweighed after being emptied, to assess the weight of the samples.

**Paracetamol experiments**

The same procedure was followed, with pressure and control groups each comprising five rats. Because of the shorter half-life of paracetamol (21), both experiments were performed during the same day. The minimum time interval between the two doses (3 h) corresponded to approximately 8–10 half-lives. The samples were transferred to 0.9 ml of an aqueous solution containing 0.9 mg of nonradioactive carrier paracetamol.

**Morphine experiments**

Both experiments were performed during the same day, and the interval between the two doses (4 h) corresponded to approximately 8 apparent half-lives of morphine (22) and resulted in plasma concentrations less than 50 µg/liter prior to the second experiment. The blood was transferred to siliconized glass tubes containing 50 µl of a 0.05% solution of nonradioactive carrier morphine.

**Analysis**

Since all samples from the pressure experiments were partially hemolyzed during the transfer out of the chamber, the analyses of antipyrine and paracetamol were performed in full blood completely hemolyzed by freezing and thawing. Complete hemolysis of the morphine samples was achieved by the addition of dipotassium hydrogen phosphate (K₂HPO₄).

Liquid scintillation counting after selective extraction of the unchanged drug was used for measurement of [¹⁴C]antipyrine (23). After addition of 0.5 ml 1 N NaOH, the samples were extracted with 5 ml chloroform using a vortex mixer for 1 min. The chloroform phase (3 ml) was transferred to a counting vial and evaporated to dryness in a stream of N₂ using gentle heat. The residue was dissolved in 6 ml of Lumagel (Lumac Systems, A.G., Basel, Switzerland) and counted.

Ethyl acetate (8 ml) was used for extraction of [¹H]paracetamol (24). Five ml of the organic phase was evaporated to dryness in a stream of N₂; 5 ml of Unisolve (Koch-Light Laboratories Ltd., Colnbrook, Bucks, U.K.) was added for liquid scintillation counting.

For the determination of [¹H]morphine, the blood sample with 200 µl K₂HPO₄ and 50 µl 0.05% carrier drug solution were extracted with 1.5 ml of ethylene chloride/amyl alcohol (70:30 v/v) (25). One ml of the organic phase was transferred to scintillation vials, evaporated to dryness, and counted after the addition of 5 ml Lumagel.

**Calculations**

The drug concentrations in whole blood were plotted on semilogarithmic time-concentration graphs. The individual half-lives in the antipyrine and paracetamol experiments were calculated from the curves by linear regression analysis. The metabolic clearance (C) was calculated using the following equation: C = (0.693/Tₕ½) • Vₐ, where Vₐ is the apparent volume of distribution determined by dividing the given dose by the extrapolated blood concentration at zero time. The statistical analysis was performed by Student's two-tailed t test.
RESULTS

The antipyrine concentrations in whole blood from the control group and the pressure group are shown in Fig. 1. The derived pharmacokinetic parameters are given in Table 1. For both groups only small and statistically not significant differences in $T_{1/2}$ were found between the first and second dose in both groups. There was considerable difference between, but not within, $V_d$ and C in each group in the crossover experiments.

The paracetamol values are shown in Fig. 2 and the derived pharmacokinetic parameters are given in Table 2. No significant differences were found within the groups or between them.

The morphine concentrations are shown in Fig. 3. No significant differences were found between or within the groups. Because the drug follows a three-compartment model, pharmacokinetic parameters could not be calculated without further extending the experiments (22).

Fig. 1. Mean concentrations in whole blood after intravenous injection of $[^{14}C]$antipyrine (15 mg/kg) at 1 ATA: control experiments (upper); pressure experiments (lower). Values after 1st injection, open circles, and after 2nd injection, open squares. Number of animals, 5.
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#### TABLE 1

**Pharmacokinetic Parameters of Antipyrine at 1 ATA and at 71 ATA in Rat**

<table>
<thead>
<tr>
<th>Expt.</th>
<th>No. of Animals</th>
<th>Injection No.</th>
<th>Pressure, ATA</th>
<th>$T_{1/2}$, min</th>
<th>$V_d$, liter/kg</th>
<th>Clearance, ml·kg$^{-1}$·min$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>71.2 ± 7.0</td>
<td>1.17 ± 0.14</td>
<td>11.4 ± 1.6</td>
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<tr>
<td>Pressure group</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>78.8 ± 4.2</td>
<td>1.22 ± 0.16</td>
<td>11.7 ± 1.4</td>
</tr>
<tr>
<td>Control group</td>
<td>5</td>
<td>1</td>
<td>71</td>
<td>86.1 ± 18.5</td>
<td>0.90 ± 0.18</td>
<td>7.4 ± 1.7</td>
</tr>
<tr>
<td>Pressure group</td>
<td>5</td>
<td>2</td>
<td>71</td>
<td>86.1 ± 18.5</td>
<td>0.90 ± 0.18</td>
<td>7.4 ± 1.7</td>
</tr>
</tbody>
</table>

Values in means ± SD.

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![Graph](image1)

**Fig. 2.** Mean concentrations in whole blood after intravenous injection of [H]paracetamol (15 mg/kg) at 1 ATA: control experiments (upper), pressure experiments (lower). Values after 1st injection, open circles, and after 2nd injection, open squares. Number of animals, 5.
### Table 2

**Pharmacokinetic Parameters of Paracetamol at 1 ATA and at 71 ATA in Rat**

<table>
<thead>
<tr>
<th>Expt.</th>
<th>No. of Animals</th>
<th>Injection No.</th>
<th>Pressure, ATA</th>
<th>$T_{1/2}$, min</th>
<th>$V_a$, liter/kg</th>
<th>Clearance, ml·kg$^{-1}$·min$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>17.2 ± 3.3</td>
<td>0.60 ± 0.19</td>
<td>24.3 ± 6.5</td>
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<tr>
<td>group</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>18.2 ± 5.6</td>
<td>0.74 ± 0.20</td>
<td>29.7 ± 8.0</td>
</tr>
<tr>
<td>Pressure</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>16.4 ± 2.8</td>
<td>0.93 ± 0.19</td>
<td>39.5 ± 7.3</td>
</tr>
<tr>
<td>group</td>
<td>5</td>
<td>2</td>
<td>71</td>
<td>14.9 ± 2.7</td>
<td>0.86 ± 0.30</td>
<td>40.0 ± 12.3</td>
</tr>
</tbody>
</table>

Values in means ± SD.

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**Fig. 3.** Mean concentrations in whole blood after intravenous injection of $[^3]H$morphine (2.5 mg/kg) at 1 ATA: control experiments (*upper*), pressure experiments (*lower*). Values after 1st injection, open circles, and after 2nd injection, open squares. Number of animals, 5.
DISCUSSION

Drug metabolism under hyperbaric conditions has been studied by Tofano (15), who found decreased renal excretion of free morphine in the urine from rats at 21 ATA, and by Kramer et al. (14), who reported an increased half-life of meperidine in dogs at 6 ATA air. The in vitro O-dealkylation of p-nitroanisole in the 9000-g liver fraction was found to be selectively increased after long-term exposure of rats to 21 ATA He-O₂ (11). Morphine metabolism in the rat liver microsomal preparations at 19.2 ATA He-O₂ has been found to be unaltered (10). However, contradictory results have been published by Tofano et al. (12), who found the N-dealkylation of morphine in the 9000-g rat liver fraction to be increased after exposure to 11 ATA He-O₂.

A previously published study (13) from our laboratory showed unchanged metabolism of diazepam, sulfadimidine, sulfanilamide, antipyrine, and paracetamol in the isolated rat liver cell suspensions at 70 ATA.

The purpose of the present investigation was to study the pharmacokinetics of antipyrine, paracetamol, and morphine in the rat at 71 ATA. The experimental protocol included injection of anesthetic drugs and surgical stress 3 h before the first experimental dose. Since this might interfere with the pharmacokinetics of the drugs, separate control groups subjected to these procedures were studied at 1 ATA.

The oxidative breakdown of antipyrine in the rat amounts to approximately 70% of its total metabolism (16). The metabolism of antipyrine has been used to assess the mixed-function oxidase system in the rat (16, 23, 26), and there is evidence for ascribing the different metabolites to separate cytochrome P-450 systems. Since there are negligible variations in the metabolism of antipyrine within individuals, and while considerable differences exist between individual rats (23), each animal was used as its own control in the present study.

A small but not statistically significant increase in the antipyrine half-life was found between the first and second injection in both groups. A small but not statistically significant decrease was found in the clearance values, while the volume of distribution was unchanged. All parameters were in accordance with those reported by other investigators (21).

Approximately 73% of paracetamol is metabolized in the rat liver by sulfate and glucuronide conjugation (17). First-order kinetics was observed in our study as reported by others (21). The obtained values were in agreement with previously published data (21), and the findings at 1 ATA and 71 ATA indicate that sulfate and glucuronide-acid conjugation, which are the main routes of metabolism in the rat, are apparently not appreciably affected by increased hydrostatic pressure.

Morphine is avidly extracted from the blood stream by the liver (19), where most of the drug is conjugated with glucuronic acid. The plasma concentration curve follows a three-exponential function (22). During our sampling period some of the morphine was still being distributed, but the hepatic elimination was nevertheless a major determinant of the concentration curve. For a high-clearance drug like morphine one would expect the blood concentration profile to be altered if increased hydrostatic pressure affected the liver circulation (27, 28). Since no significant difference in the plasma concentrations was found between the first and second morphine injection, it appears that high pressure has no appreciable effect on the overall kinetics in the phase studied.

In conclusion, the present results are in keeping with our earlier findings of unaltered drug metabolism in suspensions of isolated rat liver cells at high pressure (13). The hepatic enzymes involved in the biotransformation of antipyrine and paracetamol appear not to be affected by increased hydrostatic pressure in vitro or in vivo. Neither is there any evidence of changes in the flow-dependent elimination of morphine by high pressure.

**REFERENCES**

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