Anesthetic effect and distribution of infused pentobarbital in the rat at 71 ATA

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Aanderud L, Ursin R, Furset K. Undersea Biomed Res 1987;14(2): 93–99.—The anesthetic dose requirement and distribution of 14C-labeled pentobarbital were studied in rats at 1 ATA air and at 71 ATA He-O2. Pentobarbital was infused intravenously at a rate of 5 mg·kg⁻¹·min⁻¹. The depth of anesthesia was assessed by EEG using the burst suppression of 1-s duration (silent second) as the biological end point. The mean anesthetic dose was 45.6 and 68.0 mg·kg⁻¹ at 1 and 71 ATA, respectively, representing a 49% increase at pressure (P < 0.001). The corresponding concentrations in the brain were 81.6 and 92.3 μg·g⁻¹ (not significant). The hepatic and renal pentobarbital concentrations increased by 44 and 41%, respectively, at pressure (P < 0.001). Interindividual variations in required doses and infusion lengths made comparison between tissue pentobarbital values difficult to interpret. A second series of experiments was therefore undertaken using a subanesthetic dose of pentobarbital infused at the same constant rate for 7 min. No significant changes were found in the organ distribution of the drug at pressure. The results show that high pressure antagonizes the narcotic effect of pentobarbital and that the distribution of pentobarbital is not significantly altered in the rat at 71 ATA.

The decreased effect of anesthetic agents at increased ambient pressure has been observed in many species, including man. The barbiturates that are the most widely used drugs in anesthetic practice have been studied by several groups. Pressure antagonism has been observed for thiopental (1–4), metohexitol (3, 5), pentobarbital (6–8), phenobarbital (9–11), and barbital (8). Common to all studies was a complete reversal or reduced anesthetic effect at pressure.

The cause for this antagonism is not known, but a number of hypotheses have been put forward to explain the phenomenon (1). By measuring the brain concentration of pentobarbital in the rat during infusion at a specific and well-defined anesthetic depth, supplemented with a similar series with infusion at the same constant rate of a fixed dose, we intended to find an answer to the question: Is the decreased barbiturate
effect at high pressure due to decreased brain sensitivity or is the distribution of the drug altered?

MATERIALS AND METHODS

Male Wistar rats weighing 200–300 g were used. The implantation techniques and handling procedure have been described in detail elsewhere (4, 12). Polyethylene catheters (PE 50) were inserted into the tail artery for blood sampling and into the femoral vein for drug infusion. Both catheters were tunneled subcutaneously to the back. The 4-liter chamber was compressed at a rate of 0.3 ATA·min⁻¹ with helium, and oxygen was added to keep the partial pressure at 0.4 ATA. The animals were observed through the front port window of the chamber (4). The rectal temperature was maintained at 37.5 ± 0.5°C during the experiments, with a chamber temperature of 32–34°C by adjusting the external heat supply. The partial pressure of oxygen was monitored by a paramagnetic oxygen analyzer (Servomex DA 580, Taylor & Servomex, Crawborough, Sussex, UK). CO₂ was analyzed with CO₂ test tubes (Drägerwerke, Lübeck, W. Germany), with a detection limit of 0.1% vol/vol, and was invariably found to be below this limit (5.4 Torr). The EEG was recorded from stainless steel screws implanted in the cranium 2 wk before the experiments. After a baseline recording, the EEG was recorded from the start of the compression procedure at each 10-ATA interval for 3 min for frequency analysis. After 1 h of adaptation at 71 ATA with recording of a baseline EEG, [¹⁴C]pentobarbital (ICN Pharmaceuticals, Inc., Irvine, CA) was infused as a constant rate infusion of 5 mg·kg⁻¹·min⁻¹ via a high-pressure syringe by means of an electric pump delivering a volume of 0.1 ml·min⁻¹. The EEG was recorded on a polygraph (Grass Instruments, Quincy, MA) at a paper speed of 15 mm·s⁻¹ until burst suppression of 1-s duration (silent second) appeared (13). At this point the infusion was stopped, samples of arterial blood were collected through the sampling system, and the animals were killed instantly by rapid decompression.

The control animals were subjected to the same procedure at 1 ATA air in the pressure chamber or in a Plexiglas chamber of the same size with identical interior, including the fan. The adaptation, operative procedures, and the time of restraining in the chamber were identical for all animals in the control and pressure groups. The control animals were killed by i.v. injection of 0.3 ml saturated KCl solution. The brain, liver, kidney, muscle, and fat tissues were excised immediately and prepared for determination of the drug content.

Considerable interindividual variations in barbiturate dose requirement made the pharmacokinetic interpretation of blood and tissue radioactivity difficult. To obtain more precise information of the distribution of pentobarbital at high pressure, a second series of experiments was performed. The same constant rate infusion of a fixed subanesthetic dose (5 mg·kg⁻¹·min⁻¹) was given for 7 min in a control group (n = 10) and a pressure group (n = 9), and the tissue drug concentrations were measured.

DRUG ANALYSIS

The midlobe of the liver, the left kidney, part of the abdominal muscle, perirenal fat, and the cerebrum were cut into small pieces, transferred to glass tubes containing
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1 ml 0.9% saline/g tissue, and homogenized by the Potter-Elvehjem technique. Two hundred microliter of the homogenate was transferred to counting vials containing 1 ml Lumasolve (Lumac Systems, Basel, Switzerland). The samples were kept at room temperature until the solutions were clear and then bleached by dropwise addition of 0.5 ml of 30% H$_2$O$_2$. Finally, 10 ml of a mixture of Lumagel (Lumac Systems) and 0.5 M HCl (9:1) were added.

The blood samples were collected into heparinized, 1-ml plastic syringes, weighed, and transferred to counting vials. Lumasolve-isopropanol 1:2 was added, followed by H$_2$O$_2$ and Lumagel-HCl as described above. The samples were counted in a Hewlett Packard liquid scintillation spectrometer with channel-ratio correction for quenching.

In the second series, the tissues were dissolved in Lumasolve in the counting vials in 24 h at room temperature. Otherwise the procedure was as described above.

The Mann-Whitney test for independent data was used for the statistical analysis.

RESULTS

The induction doses of pentobarbital at 1 ATA air and 71 ATA He-O$_2$ are presented in Table 1. The mean values with SEM were 45.6 ± 2.2 and 68.0 ± 1.2 mg·kg$^{-1}$, respectively, which is a 49% increase ($P < 0.001$).

Because the drug was infused at a constant rate, the time to the appearance of the silent second increased proportionately with the induction dose from 9.11 to 13.59 min. The pentobarbital blood concentrations at silent second (Table 2) were 97.9 ±

TABLE 1

<table>
<thead>
<tr>
<th>Dose, mg/kg</th>
<th>Infusion time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ATA</td>
<td>71 ATA</td>
</tr>
<tr>
<td>48.4</td>
<td>66.0</td>
</tr>
<tr>
<td>36.7</td>
<td>69.2</td>
</tr>
<tr>
<td>37.6</td>
<td>64.2</td>
</tr>
<tr>
<td>48.9</td>
<td>65.7</td>
</tr>
<tr>
<td>42.9</td>
<td>74.3</td>
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<tr>
<td>37.2</td>
<td>72.9</td>
</tr>
<tr>
<td>46.4</td>
<td>63.5</td>
</tr>
<tr>
<td>60.5</td>
<td>67.8</td>
</tr>
<tr>
<td>52.6</td>
<td>70.4</td>
</tr>
<tr>
<td>46.9</td>
<td>65.9</td>
</tr>
<tr>
<td>43.1</td>
<td>8.62</td>
</tr>
</tbody>
</table>

Values are means ± SEM; 1 ATA air ($n = 11$); 71 ATA ($n = 10$). *$P < 0.001$
TABLE 2
PENTOBARBITAL CONCENTRATIONS (μG/G) IN WHOLE BLOOD AND VARIOUS ORGANS AT SILENT SECOND

<table>
<thead>
<tr>
<th>Pressure</th>
<th>Blood</th>
<th>Brain</th>
<th>Liver</th>
<th>Kidney</th>
<th>Muscle</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ATA</td>
<td>97.9</td>
<td>81.3</td>
<td>139.7</td>
<td>132.5</td>
<td>48.2</td>
<td>51.1</td>
</tr>
<tr>
<td>(n = 11)</td>
<td>±11.3</td>
<td>±5.1</td>
<td>±9.3</td>
<td>±7.6</td>
<td>±7.4</td>
<td>±6.5</td>
</tr>
<tr>
<td>71 ATA</td>
<td>117.6</td>
<td>92.3</td>
<td>201.1*</td>
<td>173.5*</td>
<td>38.1</td>
<td>66.4</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>±10.5</td>
<td>±3.0</td>
<td>±7.5</td>
<td>±13.4</td>
<td>±0.6</td>
<td>±7.7</td>
</tr>
</tbody>
</table>

Values are means ± SEM. *P < 0.001.

TABLE 3
PENTOBARBITAL CONCENTRATIONS (μG/G) IN WHOLE BLOOD AND VARIOUS ORGANS AFTER 7 MIN CONSTANT RATE INFUSION 5 mg·kg⁻¹·min⁻¹ AT 1 AND 71 ATA

<table>
<thead>
<tr>
<th>Pressure</th>
<th>Blood</th>
<th>Brain</th>
<th>Liver</th>
<th>Kidney</th>
<th>Muscle</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ATA</td>
<td>57.5</td>
<td>56.3</td>
<td>117.1</td>
<td>118.8</td>
<td>29.7</td>
<td>27.5</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>±3.8</td>
<td>±1.7</td>
<td>±4.3</td>
<td>±4.5</td>
<td>±1.7</td>
<td>±1.5</td>
</tr>
<tr>
<td>71 ATA</td>
<td>44.7</td>
<td>52.5</td>
<td>124.5</td>
<td>128.5</td>
<td>35.6</td>
<td>29.4</td>
</tr>
<tr>
<td>(n = 9)</td>
<td>±2.4</td>
<td>±1.6</td>
<td>±9.4</td>
<td>±10.3</td>
<td>±3.9</td>
<td>±4.2</td>
</tr>
</tbody>
</table>

Values are means ± SEM; no significant differences were observed.

11.3 at 1 ATA vs. 117.6 ± 10.5 μg·g⁻¹ at 71 ATA, or increased by 19% (not significant, n.s.).

The mean brain pentobarbital concentrations (Table 2) showed a slight (14%) but not statistically significant increase, from 81.3 ± 5.3 μg·g⁻¹ at 1 ATA to 92.3 ± 3.0 μg·g⁻¹ at 71 ATA. The corresponding brain: blood concentration ratios were 0.98 ± 0.13 and 0.82 ± 0.17 (n.s.).

The mean liver and kidney pentobarbital concentrations increased by 44 and 41%, respectively, at 71 ATA (P < 0.001). The muscle and fat contents were not significantly altered at pressure (Table 2).

Table 3 shows the blood and tissue concentrations after constant rate infusion of 5 mg·kg⁻²·min⁻¹ for 7 min. No significant changes were found.

DISCUSSION

The EEG parameter burst suppression of 1-s duration (silent second) has been shown to be a highly reproducible parameter to assess the anesthetic depth during barbiturate infusion in rats (13, 14). This criterion has been used to study the effect of thiopental in the rat at 71 ATA (4). The infusion rate of 5 mg·kg⁻¹·min⁻¹ pentobarbital was found by Bolander et al. (14) to be the dose rate that resulted in the appearance of the silent second with the lowest total dose. The EEG as a parameter
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...of anesthetic depth was chosen because this method gives a direct measure of the cerebral activity. In addition, no repetitive sensory stimulation was necessary during the experiments.

Electroencephalographic studies have been performed at increased pressure in human volunteers and in rats (4, 15, 16). The changes in EEG-frequency pattern, which previously have been described at high pressure (16), were not perceptible by visual inspection and did not disturb the identification of the silent second.

The increased dosage requirement at 71 ATA supports our earlier studies with thiopental. The result is also in keeping with other reports on the effect of barbiturates at high pressure (1–11). Irrespective of the method of end-point registration, significant pressure antagonism has been found.

It has not been established whether this antagonism is due to altered cerebral barbiturate uptake or reduced CNS sensitivity. Data suggesting decreased cerebral thiopental uptake after rapid i.v. injection in the rat at 71 ATA have previously been published by our group (17). Increased uptake of methylene blue (18) and tetracycline (19) has been found after decompression from 6 ATA in rabbits. The possibility of altered drug distribution at high pressure as a mechanism contributing to the pressure reversal of anesthesia in some situations can therefore not be excluded.

Induction of anesthesia even after constant rate infusion for 7–15 min does not result in a steady state with regard to drug distribution. This would require a prolonged infusion time (approximately 5 × t½ = 250 min) and was not possible for practical reasons. Because the ranges of infusion times were 7.33–12.10 min in the control group and 12.70–14.85 min in the pressure group, pharmacokinetic calculations from these data were difficult to evaluate. Thus, considerable latitude exists with regard to the interpretation of the drug concentrations observed when the silent second occurred.

The barbiturate concentrations were estimated from 14C scintillation counting. The drug molecule was labeled in the 2-carbon position of the barbiturate ring, which is unaffected by metabolism. Values are therefore assumed to represent the actual drug concentrations in the tissues.

Pentobarbital concentrations in the blood and tissues would be expected to increase at silent second in the pressure group, because the average infused dose was 49% higher than in the corresponding control group at 1 ATA. We found the mean brain concentrations to be only marginally increased at pressure, but with great deviations.

This may be explained by the slow equilibration of pentobarbital between blood and brain after constant rate infusion (20), which is due to the relatively low lipid solubility of the undissociated drug (21) and the considerable interindividual differences in dose requirement within both groups. Although concentrations in the liver and kidney tissues were significantly increased in the pressure experiments, the amount of infused drug was greater and these tissues contained the same fraction of the total dose (57%) in both situations.

The fixed dose infusion experiments, however, revealed no significant pharmacokinetic changes at pressure. These data are directly comparable and indicate that pentobarbital distribution is unaltered at high pressure, at least in subanesthetic doses. The brain blood flow in the awake rat has been found unchanged at 71 ATA (22). Data for anesthetized animals at high pressure are at present not available. The distribution of thiopental to the brain in the early distribution phase in the rat at 71...
ATA suggests that the brain uptake of this highly fat soluble barbiturate may be altered after an i.v. bolus injection (17).

The half-life of pentobarbital in rats is approximately 50 min (23). Possible changes in hepatic perfusion and clearance are therefore unlikely to influence the effect during the short-lasting infusion in the present experiments. Moreover, previous studies on the metabolism of several drugs at 71 ATA do not indicate altered drug metabolism at high pressure (24, 25).

Due to technical problems, EEG frequency analysis was possible only in 50% of the recordings. Statistical analysis was therefore not feasible, but the preliminary results seem to indicate a dissimilar effect of pressure on the barbiturate-induced EEG patterns. Further studies are planned in our laboratory.

In conclusion, the results confirm that higher doses of a barbiturate are required to obtain a narcotic effect at high pressure. The distribution of pentobarbital after constant rate infusion of a fixed subanaesthetic dose was not altered significantly at 71 ATA. The results lend support to the concept of a pressure antagonism of pentobarbital and suggest that this phenomenon is not caused by altered drug distribution.

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Aanderud L, Ursin R, Furset K. Effet anesthésique et distribution du pentobarbital infusé chez le rat à 71 ATA. Undersea Biomed Res 1987;14(2): 93–99. La dose d’anesthésique nécessaire et la distribution du pentobarbital marqué au carbone quatorze (14C) furent étudiées chez des rats avec de l’air à 1 ATA et avec un mélange de HeO2 à 71 ATA. Le pentobarbital fut infusé de façon intraveineuse à un taux de 5 mg·kg⁻¹·min⁻¹. Le niveau de l’anesthésie fut évalué à l’aide de l’enregistrement de EEG en utilisant la suppression de bouffées d’une durée de 1 s (seconde silencieuse) comme point final biologique. La dose anesthésique moyenne fut de 45,6 mg·kg⁻¹ et 68,0 mg·kg⁻¹ à 1 et 71 ATA, respectivement. Ceci représente une augmentation de 49% sous pression (P < 0.001). Les concentrations correspondantes dans le cerveau étaient 81,6 et 92,3 μg·g⁻¹ (ns). Les concentrations hépatique et rénale de pentobarbital augmentèrent de 44%, respectivement, sous pression (P < 0.001). Les variations inter-individuelles dans les doses nécessaires et les temps d’infusion rendirent la comparaison entre les valeurs de pentobarbital des tissus difficile à interpréter. Une seconde série d’expériences fut donc entreprise en utilisant une dose sous-anesthésique de pentobarbital infusée au même taux constant pendant 7 min. Aucun changement significatif ne fut observé dans la distribution de la substance dans les organes sous pression. Les résultats montrent que la haute pression antagonise l’effet narcotique du pentobarbital et que la distribution du pentobarbital n’est pas significativement modifiée chez le rat à 71 ATA.

electroencephalographie  pentobarbital
anesthésie renversement de la pression
hyperbare pharmacodynamie
pharmacocinétique

REFERENCES
