Binding of Thiopental to Plasma Proteins:

Effects on Distribution in the Brain and Heart

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Thiopental-¹⁴C (30 mg and 10 µCi/kg) was injected intravenously into rats 36-48 hours following bilateral nephrectomy and one minute after pretreatment with sulfadimethoxine (30 mg/kg, iv). Control groups of normal and sham-operated animals were used. The distributions of radioactivity in plasma, brain, and heart 1, 5, and 30 minutes after injection were examined. Uremic and sulfonamide-pretreated rats showed significantly higher levels of ¹⁴C in brain and heart and more free thiopental in plasma at each time than did control animals. There was a significant correlation between the free thiopental in plasma and total drug concentrations in the brain and heart. Uremic rats bound less thiopental in plasma compared with controls in spite of normal total plasma protein and albumin concentrations. It is concluded that reduced protein binding of thiopental leads to the accelerated distribution and increased drug concentrations in the brain and heart. (Key words: Anesthetics, intravenous, thiopental; Protein, binding, thiopental; Kidney, uremia, thiopental.)

We previously described the plasma protein binding of thiopental in healthy subjects and in patients with impaired renal function. In healthy volunteers 28.0 ± 0.9 per cent of thiopental in the plasma was unbound, while in uremic patients 55.7 ± 1.5 per cent remained free. We speculated that a reduction in plasma protein binding would hasten, augment, and prolong the hypnotic and cardiovascular effects of thiopental. Chaplin and colleagues found that intravenous administration of high doses of aspirin, phenylbutazone, or naproxen to rats that had just regained the righting reflex after an anesthetic dose of thiopental caused reappearance of sleep. Csögőr and Kerék demonstrated in patients that prior administration of sulfa-"ine" reduced the hypnotic and anesthetic doses of thiopental, as well as reactions to painful stimulation during carotid angiography. Both groups of investigators suggested that their results were due to competitive displacement of thiopental from its binding sites on albumin, thus increasing the concentration of free drug available for diffusion into the tissues. Direct evidence that a decrease in plasma protein binding of thiopental increases its distribution into tissues has not been available, however. We conducted experiments in rats that had undergone bilateral nephrectomies and treatment with sulfadimethoxine to examine further the plasma protein binding and tissue distribution of thiopental.

Methods

Male Charles River rats, weighing 150-200 g, were used. The animals came from the same source and were given normal laboratory food ad libitum. A period of at least 24 hours was always allowed after transportation before carrying out the experiments. Four groups of animals were used:

I. Normal.

II. Sulfonamide pretreated: sulfadimethoxine (Madribut) was injected in a dose of 30 mg/kg, iv one minute before thiopental administration.

III. Uremic: bilateral nephrectomies were performed through an anterior abdominal incision under light ether anesthesia.

IV. Sham-operated: the same incision and

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closures procedure used for bilateral nephrectomies was performed without removing the kidneys.

Experiments on nephrectomized and sham-operated rats were carried out 36–48 hours postoperatively. The normal group served as the control for the animals pretreated with sulfonamide and the sham-operated group was the control for the uremic animals.

Thiopental-14C,‡‡ specific activity 8.8 mCi/mM, was mixed with the unlabeled drug.§§ The chemical purity of the labeled drug was checked by thin-layer chromatography using SiO₂ plates in a benzene, methanol, and acetic acid system in 45:8:4 v/v proportions, followed by scanning on a Packard Radiochromatogram scanner. No trace of impurity was found. Thiopental-14C was injected intravenously using a dose of 30 mg/kg and 10 μCi/kg. The animals were decapitated, in groups of five (normal and sulfonamide-pretreated) and ten (uremic and sham-operated), 1.5, and 30 minutes following injection. Blood was immediately collected in heparinized tubes and portions of the brain and heart were excised. The samples were accurately weighed and then placed in scintillation counting vials. NCS solubilizer,** 0.75 ml was added and the vials were heated on a slide warmer at a temperature not exceeding 50°C. After the tissue was completely solubilized, 10 ml of counting solution*** were added to each vial and radioactivity was measured in a Packard Model 3310 Tricarb liquid-scintillation spectrometer. All measurements were made in triplicate. Quench corrections were made using the channels-ratio method.

Plasma was separated from the blood; 0.1 ml was used to measure the total thiopental concentration and a 1-ml sample was dialyzed against phosphate buffer to measure the protein-bound fraction (see below). Counting was done as with the tissue samples.

In addition, the following experiments were conducted:

‡‡ ICN—Isotope and Nuclear Division, Irvine, California.
** Amersham-Searle Corporation, Arlington Heights, Illinois.
*** 5.5 g PPO, 0.1 g POPP, 200 ml Triton X-100 and 800 ml toluene.

### PLASMA PROTEIN BINDING AND PLASMA PROTEINS, BUN, AND CREATININE

The plasma protein binding of thiopental, total plasma proteins, albumin, BUN, and creatinine were measured in blood taken from ten normal and ten uremic rats. For measuring plasma protein-binding capacity, thiopental was added to plasma in one compartment of an equilibrium dialysis cell to produce a concentration of 10 μg/ml; the other compartment contained Sorensen’s 0.1-M phosphate buffer, pH 7.4. In a duplicate experiment, thiopental was added to the buffer side of the cell. A cellulose membrane separated the two compartments, and equilibrium dialysis was carried out for 24 hours at 4°C.

Thiopental was extracted from plasma and buffer after dialysis and its concentration measured using an Aminco-Bowman spectrophotofluorometer as previously described.†† Protein binding was calculated from the difference between concentrations of the drug on the two sides of the dialysis cell. Plasma proteins, albumin, urea nitrogen, and creatinine were measured by AutoAnalyzer methods.

### THIOPENTAL SLEEP TIMES

Two groups of nephrectomized and control rats, each consisting of five animals, were used. Nephrectomized animals were tested 36–48 hours following the operation. Thiopental sodium in a dose of 30 mg/kg was injected intraperitoneally in a thermally controlled room. Duration of anesthesia was taken as the interval between the time of administration and the recovery of the righting reflex. In each instance, the recovery of the righting reflex was checked by placing the rat on its back once again and the end point recorded when the animal regained position with all feet on the ground.

### Results

**THIOPENTAL SLEEP TIME**

Results were analyzed by the Wilcoxon rank-sum test. Nephrectomized rats slept 287.2 minutes, whereas control rats slept 60 minutes, P < .001.
PLASMA PROTEIN BINDING OF THIOPENTAL,
TOTAL PLASMA PROTEINS, ALBUMIN,
BUN, AND CREATININE

Results of the plasma protein binding studies in vitro were analyzed by the two-sample (unpaired) t-test. Plasma from nephrectomized rats showed a significant reduction in binding of thiopental to the proteins, P < .001. At a thiopental concentration of 10 μg/ml, 43.2 per cent of the drug was bound in uremic plasma, compared with 56.2 per cent in the normal plasma (table 1). Hotelling's T² procedure¹ revealed overall significant differences, P < .0003, between the nephrectomized and normal rats. Univariate follow-up comparisons indicated that the differences between the two groups resulted from differences in BUN, P < .0001, and creatinine, P < .0002, values. There was no significant difference in total protein or albumin values between the two groups (table 1).

LEVELS OF THIOPENTAL IN HEART,
BRAIN, AND PLASMA

A two-factor (groups and times) multivariate analysis of variance was performed on the results. There were significant differences among groups (normal, sulfonamide-pretreated, sham-operated, and uremic), P < .0001, and time levels (1, 5, and 30 minutes following administration), P < .0001. Further analysis revealed a significant, P < .001, linear decrease in radioactivity (thiopental plus metabolites) levels in heart, brain, and plasma over the time interval studied. A pair-wise comparison between the normal and sulfonamide-pretreated groups showed that they differed significantly, P < .0001, with respect to levels of radioactivity present in the heart, brain, and plasma at 1, 5, and 30 minutes following administration. A similar pair-wise comparison between the sham-operated and uremic groups showed the same significant difference, P < .0001. The means and standard deviations for each of the four groups at each of the time levels are shown in table 2. There was a significant correlation, P < .001, between the free thiopental level and thiopental concentrations in the heart, r = .87, and brain, r = .82.

Discussion

At the time of our experiments, nephrectomized rats were severely uremic but had normal total protein and albumin values. In spite of the normal protein values, uremic rats showed decreased plasma protein binding of thiopental. This corroborates our previous observation¹ that there is a change in thiopental protein binding during renal insufficiency that is not the result of altered serum albumin levels. Uremic rats slept four times longer than normal rats after a dose of thiopental. This corroborates the similar increase in sensitivity to the hypnotic effect of barbiturates reported to occur in uremic patients.²

We postulated that if the potentiation of the barbiturate effect were due to reduced plasma protein binding and increased tissue distribution of the drug, then the interaction should be mimicked by pretreatment with a drug known to alter thiopental binding to plasma. Sulfadimethoxine is a highly plasma protein-bound drug,³ which we found in preliminary experiments to reduce the plasma protein binding of thiopental significantly, P < .001.
Table 2. Radioactivity (Thiopental, μg/g Tissue or μg/ml Plasma) in the Heart, Brain, and Plasma of the Rat after Administration of Thiopental-$^{14}$C

<table>
<thead>
<tr>
<th></th>
<th>Heart (%)</th>
<th>Brain (%)</th>
<th>Total (%)</th>
<th>Bound (%)</th>
<th>Unbound (%)</th>
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<tr>
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<tr>
<td>1 minute after administration</td>
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</tr>
<tr>
<td>Normal rats</td>
<td>25.76 (± 7.25)</td>
<td>21.80 (± 6.70)</td>
<td>1.85 (± 0.65)</td>
<td>1.45 (± 0.58)</td>
<td>0.40 (± 0.08)</td>
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<tr>
<td>Sulfonamide-pretreated rats</td>
<td>65.50 (± 33.99)</td>
<td>54.92 (± 31.44)</td>
<td>4.05 (± 1.82)</td>
<td>2.13 (± 0.70)</td>
<td>1.92 (± 1.15)</td>
</tr>
<tr>
<td>Sham-operated rats</td>
<td>31.26 (± 7.37)</td>
<td>29.32 (± 1.71)</td>
<td>2.73 (± 0.60)</td>
<td>2.01 (± 0.52)</td>
<td>0.54 (± 0.11)</td>
</tr>
<tr>
<td>Uremic rats</td>
<td>46.82 (± 19.48)</td>
<td>34.28 (± 11.59)</td>
<td>2.02 (± 0.96)</td>
<td>1.05 (± 0.59)</td>
<td>1.00 (± 0.61)</td>
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<td>5 minutes after administration</td>
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<tr>
<td>Normal rats</td>
<td>17.66 (± 1.49)</td>
<td>16.50 (± 10.14)</td>
<td>1.50 (± 0.14)</td>
<td>1.18 (± 0.15)</td>
<td>0.32 (± 0.05)</td>
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<tr>
<td>Sulfonamide-pretreated rats</td>
<td>39.32 (± 5.04)</td>
<td>38.28 (± 4.31)</td>
<td>2.24 (± 0.35)</td>
<td>1.09 (± 0.25)</td>
<td>1.15 (± 0.15)</td>
</tr>
<tr>
<td>Sham-operated rats</td>
<td>27.76 (± 5.06)</td>
<td>23.08 (± 5.63)</td>
<td>2.56 (± 1.01)</td>
<td>2.07 (± 0.70)</td>
<td>0.58 (± 0.37)</td>
</tr>
<tr>
<td>Uremic rats</td>
<td>39.62 (± 4.86)</td>
<td>36.26 (± 5.63)</td>
<td>1.86 (± 0.60)</td>
<td>0.80 (± 0.27)</td>
<td>0.88 (± 0.27)</td>
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<tr>
<td>30 minutes after administration</td>
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<tr>
<td>Normal rats</td>
<td>15.08 (± 1.53)</td>
<td>11.50 (± 2.18)</td>
<td>1.40 (± 0.27)</td>
<td>1.12 (± 0.21)</td>
<td>0.28 (± 0.07)</td>
</tr>
<tr>
<td>Sulfonamide-pretreated rats</td>
<td>35.63 (± 7.84)</td>
<td>25.06 (± 2.30)</td>
<td>2.10 (± 0.27)</td>
<td>1.14 (± 0.18)</td>
<td>0.96 (± 0.12)</td>
</tr>
<tr>
<td>Sham-operated rats</td>
<td>17.88 (± 2.21)</td>
<td>13.88 (± 0.98)</td>
<td>2.32 (± 0.95)</td>
<td>2.03 (± 1.12)</td>
<td>0.49 (± 0.25)</td>
</tr>
<tr>
<td>Uremic rats</td>
<td>27.63 (± 8.08)</td>
<td>22.56 (± 6.11)</td>
<td>1.87 (± 0.50)</td>
<td>0.61 (± 0.29)</td>
<td>0.84 (± 0.23)</td>
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</table>

* Total plasma levels were measured for all rats in each treatment group and each time interval. Unbound and bound plasma levels, however, were measured for only five of the ten rats in the sham-operated and uremic groups at each of the times.

† Mean and SD (in parentheses).

binding of thiopental by a magnitude similar to that of the reduction produced by uremia. The data obtained in the present study indicate that alteration of the plasma protein binding by another drug can increase tissue levels of total thiopental by an amount similar to that observed in uremic animals. This supports our hypothesis that uremia causes a potentiation of thiopental anesthesia through an effect on the plasma protein binding of the barbiturate.

The reported tissue levels of the drug include thiopental metabolites. The contribution of metabolites should not be of concern, especially 1 and 5 minutes after injection, because our own results (unpublished) confirm the work of others demonstrating that metabolism of the drug is extremely slow. It is already established that redistribution rather than metabolism is responsible for the decline in thiopental levels at these early times.

More drug was found in the tissues of uremic and sulfonamide-pretreated animals compared with sham-operated and normal controls. The increased tissue distribution of the drug in the
uremic and sulfonamide-pretreated animals may be explained by a reduction in drug plasma binding, since there is a strong correlation between tissue levels and plasma levels of free drug. It seems unlikely that the similar effects on drug distribution caused by uremia and by sulfonamide are the results of factors other than their similar effects on the plasma protein binding of thiopental. For example, it is unlikely that the higher tissue concentrations of thiopental in uremic and sulfonamide-treated animals are due to an inhibition of drug metabolism. An influence of thiopental metabolism on the anesthetic course and early tissue levels of the drug is minimal. In addition, sulfonamides do not inhibit pentobarbital metabolism by liver homogenates. One could anticipate that a decrease in the plasma binding of thiopental may even increase its rate of metabolism because of a higher concentration of the drug in the tissues involved in metabolism.

We conclude from our results that the reduced plasma protein binding of thiopental caused by uremia and sulfonamide pretreatment justifies the assumption of an accelerated distribution and increased drug concentration in the brain and heart. A more rapid onset of action and an enhanced activity of thiopental are logical consequences. Since pharmacologic effects are directly related to the unbound drug in plasma, potentiation of thiopental action would also be expected under other conditions where binding of the barbiturate to plasma proteins may be reduced by changes in the proteins, either quantitatively or qualitatively, or by other drugs that compete for protein binding sites.

References