Acute Tolerance to Thiopental in Canine Cerebral Oxygen Consumption Studies

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The response of cerebral O₂ consumption rate (CMR₀₂) to thiopental was studied in three groups of unpremedicated dogs at 37.0° C during light halothane anesthesia (0.8 per cent expired). CMR₀₂ was calculated from direct measurements of cerebral blood flow and the arterial-sagittal sinus blood O₂ content difference. One group received a thiopental injection of 10 mg/kg, which depressed CMR₀₂ 9 per cent at one minute, with return to control within one hour. Two other groups received constant infusions of thiopental (23 mg/kg/hr) and were studied for two hours. One of the latter groups had pretreatment, two hours prior to infusion, with 10 mg/kg thiopental. After two hours of infusion, CMR₀₂ was reduced 17 per cent in the pretreated group and 40 per cent in the infusion-only group. At this time, arterial and CSF thiopental concentrations were higher in the pretreated group than in the infusion-only group. These results confirm a parallelism between functional and metabolic aspects of acute CNS tolerance to thiopental. (Key words: Thiopental; Cerebral metabolism; Acute tolerance.)

Thiopental has been shown to reduce cerebral O₂ consumption rate (CMR₀₂) in both man¹,² and experimental animals.³,⁴ Functional tolerance to thiopental, both acute and chronic, has been observed in man ⁵ and dogs.⁶⁻⁹ The present study explores the possibility of a parallelism between the cerebral metabolic response and the functional response to thiopental in dogs by comparing the response of CMR₀₂ with plasma and cerebrospinal fluid (CSF) concentrations of thiopental, with and without pretreatment with thiopental. The findings indicate that acute tolerance to thiopental includes a metabolic component.

Methods

Unpremedicated, fasting dogs, 6 months to 3 years old, weighing 14 to 22 kg, were studied. Anesthesia was induced and maintained with halothane (Fluothane) § in 30 per cent oxygen and nitrogen. Succinylcholine, 20 mg, was administered intravenously before placement of a cuffed tracheal cannula and, thereafter, at 150 mg/hr. Cannulae were placed in the femoral artery for blood sampling and pressure determinations and in the femoral vein for infusion of drugs and blood. The dog was clipped and placed in the prone position in a water bath with the head and back above water level. Ventilation was adjusted to maintain PaCO₂ between 35 and 45 mm Hg. Inspired halothane was adjusted as required to result in an expired halothane concentration of 0.80 ± 0.05 per cent. Hemoglobin concentration was adjusted to approximately 12 gm/100 ml by withdrawal of blood and replacement with 6 per cent dextran. ¶

The blood drawn was used for preparation of thiopental standards and replacement of blood loss.

Cerebral blood flow was measured by a direct method, as previously described.¹⁰ The technique includes cannulation of the sagittal sinus and diversion of blood flow to an external collection and reinfusion system. The

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§ Kindly supplied by Ayerst Laboratories, New York.
¶ Kindly supplied by Abbott Laboratories, North Chicago, Ill.
Table 1. Description of Groups in Study of Cerebral Metabolic Response to Thiopental

<table>
<thead>
<tr>
<th>Group</th>
<th>Thiopental Schedule</th>
<th>Number of Dogs</th>
<th>Thiopental (mg/kg)</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection</td>
<td>Single injection</td>
<td>5</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Infusion</td>
<td>Continuous infusion</td>
<td>5</td>
<td>23/hour</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Injection−infusion</td>
<td>Continuous infusion two hours after single</td>
<td>5</td>
<td>23/hour</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>10 mg/kg injection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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</table>

Collected blood represents venous drainage from the anterior, superior, and lateral portions of both cerebral hemispheres (43 percent of total brain weight). The temperature of the water bath was adjusted to maintain an epidural temperature (thermistor) of 37.0 ± 0.1 C. CSF samples were obtained via a needle in the cisterna magna, left open to avoid development of excessive CSF pressure. $P_{O_2}$, $P_{CO_2}$, pH, and sagittal sinus blood $P_{O_2}$ ($P_{ssO_2}$) were determined by electrodes (IL) at 37.0 C. Arterial pressure was transduced by a strain gauge. Halothane concentrations were determined with an infrared analyzer. Hemoglobin (Hb) and oxyhemoglobin ($HbO_2$) concentrations were measured in an Instrumentation Laboratories co-oximeter, Model 182. Oxygen content was calculated from Hb, $HbO_2$, and $P_{O_2}$ in the usual manner. CMR$_O_2$ was obtained from the product of cerebral blood flow (CBF) and the arterial-sagittal sinus blood $O_2$ content difference.

Plasma and CSF thiopental concentrations were determined spectrophotofluorometrically after a double-extraction process. The accuracy of the method was 0.1 mg/liter of thiopental and was not altered by hemolysis. Thiopental standards were prepared as the sodium salt from the acid form and reported as the acid. All determinations were done with a blank and internal standard. Self-quenching required dilution for thiopental concentrations above 2.5 mg/liter.

Three groups of five dogs each were studied before and with intravenous thiopental (table 1). One group (injection group) received 10 mg/kg of thiopental in a 5-sec period. Another group (infusion group) was given 10 mg/kg at a constant rate (23 ± 1 mg/kg/hr). The third group (injection−infusion group) was given a thiopental injection of 10 mg/kg prior to the surgical preparation and, two hours later, thiopental infusion of 23 ± 1 mg/kg/hr. Both infusion groups were exposed to Kindly supplied by Abbott Laboratories, North Chicago, Ill.

Table 2. Effects of Thiopental on Canine Cerebral Metabolism and Circulation

<table>
<thead>
<tr>
<th>Group*</th>
<th>Time (min)</th>
<th>CMR$_O_2$ (ml/min/100 gm)</th>
<th>CBF (ml/min/100 gm)</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>CVR (mm Hg/ml/min/100 gm)</th>
<th>$P_{ssO_2}$ (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
<td>Mean SE</td>
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<tr>
<td>Injection</td>
<td>0</td>
<td>4.38 0.14</td>
<td>75 4</td>
<td>89 3</td>
<td>1.2 0.1</td>
<td>45 1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4.01† 0.12</td>
<td>59† 2</td>
<td>81 5</td>
<td>1.4 0.1</td>
<td>41 2</td>
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<tr>
<td></td>
<td>60</td>
<td>4.38 0.09</td>
<td>62† 3</td>
<td>102† 5</td>
<td>1.6† 0.1</td>
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<tr>
<td>Infusion</td>
<td>0</td>
<td>4.82† 0.11</td>
<td>74 5</td>
<td>104 2</td>
<td>1.4 0.1</td>
<td>45 3</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>4.60† 0.21</td>
<td>62† 6</td>
<td>106 8</td>
<td>1.7 0.1</td>
<td>42 3</td>
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<td></td>
<td>120</td>
<td>2.89† 0.19</td>
<td>36† 3</td>
<td>80† 8</td>
<td>2.3 0.3</td>
<td>37† 2</td>
</tr>
<tr>
<td>Injection−infusion</td>
<td>0</td>
<td>4.75 0.19</td>
<td>73 7</td>
<td>92† 4</td>
<td>1.3 0.1</td>
<td>43 3</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>4.48† 0.23</td>
<td>63 10</td>
<td>102 6</td>
<td>1.7† 0.2</td>
<td>42 2</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>3.95† 0.20</td>
<td>52† 5</td>
<td>84 7</td>
<td>1.6† 0.1</td>
<td>38 3</td>
</tr>
</tbody>
</table>

* See table 1 for thiopental schedule.
† Significantly different ($P < 0.05$) from control value (0 min) of each group by t test, paired data.
‡ Significantly different ($P < 0.05$) from infusion group at equivalent times by t test, unpaired data.
to halothane for two hours prior to the start of the thiopental infusion. At autopsy, the brains were weighed and examined for evidence of extracerebral contamination of blood.

Dose-response regression equations and correlation coefficients were calculated by the method of least squares. Significance of difference in mean values was tested by Student's $t$ test for paired data within each group and for unpaired data between groups.

Results

With a single injection of 10 mg/kg thiopental (injection group), mean $\text{CMRO}_2$ decreased a maximum of 9 per cent within one minute and thereafter returned to the control value within 40 to 60 minutes (table 2, fig. 1). In this group, plasma thiopental concentrations peaked in the first five minutes and thereafter progressively decreased, reaching a level at 60 minutes of approximately one third that observed at five minutes (fig. 1). The 60-minute thiopetal concentration (6 mg/l) was, in this circumstance, without apparent effect on $\text{CMRO}_2$. With infusion of 23 mg/kg/hr of thiopental (infusion group), $\text{CMRO}_2$ decreased progressively (table 2) as plasma and CSF thiopental concentrations increased (fig. 2). In this group, after 150 minutes of infusion, the mean $\text{CMRO}_2$ was reduced 40 per cent at a mean plasma thiopental concentration of 47 mg/l and a mean CSF concentration of 13.1 mg/l. When a single injection of 10 mg/kg of thiopental preceded infusion (injection-infusion group), $\text{CMRO}_2$ also decreased progressively with infusion (table 2) as plasma and CSF thiopental concentrations increased (fig. 2). However, in this group, mean $\text{CMRO}_2$ at 120 minutes was decreased only by 17 per cent despite the presence of higher mean plasma and CSF thiopental concentrations (55 and 17 mg/l, respectively). This difference between the responses of $\text{CMRO}_2$ to thiopental in the two groups is also apparent when individual $\text{CMRO}_2$ values are plotted against total amounts of thiopental infused (fig. 3 and 4). The correlation coefficients for the infusion group and the injection-infusion group are similar (−0.71 and

![Fig. 1. Canine $\text{CMRO}_2$ and plasma thiopental concentrations after intravenous injection of 10 mg/kg thiopental in 5 sec.](image1)

![Fig. 2. Canine plasma and cerebrospinal fluid thiopental concentrations during continuous intravenous infusion of 23 mg/kg/hr thiopental. Dashed lines connect mean values in the group receiving intravenous injection of 10 mg/kg thiopental two hours prior to infusion.](image2)
The slopes of the two regression lines are significantly different \( P < 0.05 \). A similar difference was present in the slopes of the regression lines calculated for CMRO\(_2\) and the plasma or CFS thiopental concentrations.

In each group, the observed reduction in mean CMRO\(_2\) was accompanied by a comparable decrease in mean CBF (table 2). Mean arterial blood pressure remained at or above 80 mm Hg in each dog. In each group, a moderate increase in mean cerebral vascular resistance occurred with time (table 2). There was no significant difference within or between the groups in Pa\(_{O_2}\), Pa\(_{CO_2}\), pH, and buffer base (not tabulated). No evidence of extracerebral contamination of blood or other cerebral vascular anomalies was found at autopsy.

**Discussion**

Thiopental is a known depressant of cerebral metabolism,\(^{1-4}\) a relationship between dose and degree of cerebral metabolic depression having been demonstrated in dogs \(^4\) and monkeys.\(^5\) Further, studies of functional activity have established that plasma thiopental concentrations and functional changes in the central nervous system are closely related after a single injection.\(^6-9\) However, after repeated injections, acute functional tolerance to thiopental develops in man and dogs. Evidence for tolerance exists in various forms. An increase in sleeping time occurs after repeated injections, and awakening occurs with progressively increased plasma thiopental concentrations.\(^6-7\)\(^,\)\(^,\)\(^12\) No relationship exists between plasma thiopental concentrations and clinical depth of anesthesia.\(^5\)\(^,\)\(^12\) Other functional responses,\(^7\)\(^,\)\(^8\) or electroencephalographic patterns.\(^12\) Further, in a study of the use of larger doses of thiopental in England than in America for comparable periods of anesthesia, it was found that awakening times did not correlate with the plasma concentrations or the total dose, and plasma thiopental concentrations were greater at the time of awakening after a large initial dose than after a smaller initial dose.\(^5\) Similarly, in dogs, if the initial dose is given in increments, the duration of narcosis is more prolonged than that after a single rapid injection.\(^6\) A pause of even ten seconds during injection alters the dose–response relationship.\(^14\)

The degree to which the acute functional response to thiopental is paralleled by the cerebral metabolic response to thiopental was not predictable from previous studies. The basic design of the present study evolved from an examination of the above reports and our pilot studies. The dose selected for the injection group (10 mg/kg) is the minimal anesthetic dose for dogs \(^15\) and, in studies by others,\(^6,14,15\) produced a short period of sleep, with return of the righting reflex in less than an hour. Acute functional tolerance to this dose has been demonstrated in dogs.\(^6\) In our study, the 10 mg/kg dose of thiopental produced an initial modest decrease in CMRO\(_2\) with return to control within an hour, despite the presence of significant plasma and CSF thiopental concentrations. Thus, the known functional effects of this dose correlate well with the observed metabolic effects. The dose selected for the infusion group (23 mg /kg/hr) resulted, in two hours, in the administration of a total amount of thiopental which has been said to be the maximally tolerated dose for dogs (45 mg/kg).\(^12\) With this infusion, the decrease in CMRO\(_2\) correlated progressively with both total dose and plasma and CSF concentrations of thiopental. The third group (injection–infusion) received both the initial injection of 10 mg/kg and, after two hours, infusion of 23 mg/kg/hr for two hours. The two-hour delay between injection and infusion permitted a return of control CMRO\(_2\) values, exceeded the time known to be necessary for return of normal function, and resulted in only minimal remaining plasma and CSF thiopental concentrations prior to infusion. The depression of CMRO\(_2\) in response to thiopental infusion in this group was significantly less than that observed in dogs not pretreated. In addition, this diminished response was observed in the presence of higher plasma and CSF thiopental concentrations relating to the initial injection. Thus, pretreatment known to produce acute cerebral functional tolerance \(^6\) in a similar manner modified the cerebral metabolic response to thiopental, and a paral-
lelism between CNS functional and metabolic responses to thiopental was confirmed. The extent to which the use of halothane as a primary anesthetic agent modified or contributed to the response observed with thiopental in this study is unknown. Such an effect, if present, was presumably present in all dogs.

The basis for acute tolerance has not been established in this or in previous studies. It does seem, however, that development of acute tolerance is somehow related to peak levels of thiopental or to duration of exposure to thiopental. Tolerance does not appear to be related to an altered rate of metabolic breakdown of thiopental, since plasma levels decline at almost identical rates after different doses. Further, in the present study, the rates of change of plasma concentrations in the infusion groups were similar. An alteration in permeability seems ruled out by the absence of any appreciable blood–brain barrier for thiopental in the dog and the lack of difference in the ratios of CSF to plasma thiopental in the present study, with and
without thiopental pretreatment. Clarification of the fundamental mechanism involved in acute tolerance probably must await basic studies of the cellular responses of the central nervous system to thiopental.

This study adds to a growing body of knowledge demonstrating the lack of a consistent pattern of change in $\text{CMRO}_2$ with various anesthetics. Each anesthetic agent studied seems to have peculiarities in the effect on $\text{CMRO}_2$ which preclude generalizations. The present study, while confirming the cerebral metabolic depressive effects of thiopental, points up the profound effect of pretreatment on the degree of decrease in $\text{CMRO}_2$ with a given dose or at a given blood or CFS level of thiopental. While this effect has not been noted to occur with inhalation anesthetics, the response of $\text{CMRO}_2$ to the latter differs in several other important respects. In dogs, $\text{CMRO}_2$ increases 11 per cent with 70 per cent nitrous oxide but decreases 17 per cent with 1 per cent halothane. Doubling the alveolar halothane concentration results in only small, insignificant further decreases in $\text{CMRO}_2$. With progressive increases in cyclopropane concentrations in man, $\text{CMRO}_2$ decreases initially, but returns to control values at greater concentrations. We conclude that cerebral metabolic responses established for one anesthetic agent do not necessarily apply to another, and that generalizations about the interrelationships of anesthesia, cerebral metabolism, and function are not appropriate at the present time.

References