Local Cerebral Glucose Utilization in Stimulated Rats Sedated with Thiopental

David P. Archer, M.D., F.R.C.P.C.,† Jennifer Froelich, M.D.,‡ Michael McHugh, M.Sc.,§ Hanna M. Pappius, Ph.D.¶

Background: Recent studies have suggested that supraspinal structures are involved in barbiturate-induced enhancement of nociceptive processing. The goal of the study was to determine whether cortical and subcortical regions involved in nociception were relatively activated or depressed by noxious stimulation during infusion of small doses of thiopental.

Methods: Local cerebral glucose utilization (LCGU) was measured with the 14C-deoxyglucose radioautographic technique in 14 rats. During the LCGU experiment, pressure was applied to the tail every 2 min, and the somatic motor response threshold was recorded. Seven animals received thiopental infusions to produce a steady-state plasma concentration (target concentrations of 10 μg/ml), and seven untreated animals served as controls.

Results: A steady-state plasma thiopental concentration (11.1 ± 1.8 to 13.0 ± 2.1 μg/ml) was accompanied by a decrease in the somatic motor response threshold from 277 ± 32 g (before thiopental) to 215 ± 41 g (P < 0.001). The somatic motor response threshold remained unchanged in the control group.

Average LCGU was 29% less in the thiopental-treated animals than in the untreated controls (P < 0.001). In cortical regions associated with nociception, LCGU was relatively increased (+3% ± 14%) during the thiopental infusion in comparison to the visual and auditory cortices (−18% ± 15%; P < 0.001). Individual structures that showed relative changes during thiopental infusion included the nucleus accumbens (+17%, P < 0.05) and the habenula (−17%, P < 0.05). Heterogenous relative changes (P < 0.05) in LCGU were observed in the auditory system: auditory cortex (−22%), medial geniculate (−16%), lateral lemniscus (+26%), superior olive (−38%).

† Associate Professor, Department of Anaesthesia, University of Calgary.
‡ Resident in Anaesthesia, Department of Anaesthesia, University of Calgary.
§ Research Assistant, Department of Neurochemistry, Montreal Neurological Institute, McGill University.
¶ Professor, Department of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University.

Received from the Department of Anaesthesia, University of Calgary, Canada, and the Department of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, Canada.

Preparation for Autoradiography

We conducted these experiments in partial restraint to minimize motion artifacts in the rat. 10 A rat was anesthetized with a cocktail of inhalation anesthetics administered into the tracheal cannula. 10 The rat was then monitored with a rectal temperature maintained at 36–38°C with a heat lamp, and the muscle tone was evaluated by visual inspection and withdrawal of mechanical, thermal, and chemical stimuli. Measurements of these variables were normal limits for our laboratory: 75 mm Hg arterial C02, 45 mm Hg arterial pH 7.30–7.45, and 90–120 mm Hg arterial pressure.

Thiopental was administered by an infusion pump to produce a steady-state plasma concentration of 10 μg/ml. The rates of infusion were adjusted until the arterial blood levels of thiopental were maintained at a level of approximately 30%. The small changes to the blood levels of thiopental were accomplished by adjusting the infusion rate of the pump. After all the animals were anesthetized, the thiopental was stopped, and the rate of the infusion pump was adjusted to maintain a steady-state plasma concentration of 10 μg/ml. The rats were then allowed to stabilize for 20 min before the experiment was started.

Measurement of Somatic Throttle Sensitivity

A modification of the paw-pulse reflex was used to measure the somatic throttle sensitivity to mechanical stimulation. The paw-pulse reflex was elicited by applying a series of taps to the plantar surface of the paw with a calibrated force transducer. The force transducer was connected to a computer and monitored continuously. The force transducer was calibrated at 0.5 N, 1 N, 2 N, and 3 N. The rat was then allowed to stabilize for 20 min before the experiment was started.

Conclusions: Nociceptive stimulation during low-dose thiopental infusions relatively increased LCGU in corticospinal regions involved in processing nociceptive inputs. The mechanisms of this effect remain controversial. 10 Because barbiturates depress sensitivity to noxious stimuli, we sought to determine whether thiopental affected the sensitivity to nociceptive stimuli in vivo. We found that thiopental increased the sensitivity to mechanical noxious stimuli in the rat. 10 The results of these experiments suggest that thiopental may be a useful tool for the study of nociceptive processing in the rat.

Materials and Methods

After approval by the Animal Care Committee of the University of Calgary, 14 male Sprague-Dawley rats weighing 250–300 g were housed in a temperature-controlled environment (22°C) with a 12-h light-dark cycle. The rats were divided into two groups: the thiopental-group and the control-group. The rats were housed individually in stainless steel cages and had free access to water and food. The rats were weighed daily and their body weights were recorded. The rats were accustomed to handling and the experimental procedures for 1 week before the experiment.

The rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (Nembutal, 35 mg/kg) and placed on a heated pad to maintain body temperature at 36–38°C. The rats were then placed in a stereotaxic frame and the skin was incised to expose the brain. A craniotomy was performed to expose the brain, and a small piece of bone was removed to allow the placement of the electrode. The electrode was inserted into the brain and the position of the electrode was verified by electrical stimulation. The rats were then allowed to stabilize for 20 min before the experiment was started.

The rats were then anesthetized with an intravenous injection of thiopental (20 mg/kg) and placed on a heated pad to maintain body temperature at 36–38°C. The rats were then placed in a stereotaxic frame and the skin was incised to expose the brain. A craniotomy was performed to expose the brain, and a small piece of bone was removed to allow the placement of the electrode. The electrode was inserted into the brain and the position of the electrode was verified by electrical stimulation. The rats were then allowed to stabilize for 20 min before the experiment was started.

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Effects of Stimulation on LC-GU During Sedation

Calgary, 14 male Sprague Dawley rats were studied. In these experiments, we prospectively measured LC-GU during repeated noxious stimulation in animals receiving sedation with steady-state infusions of thiopental and compared these measurements with concurrent control animals not receiving thiopental.

Preparation for Autoradiographic Studies

We conducted these experiments under the conditions of partial restraint commonly used for autoradiographic studies in the rat. Briefly, after weighing, each animal was anesthetized with 2% halothane in oxygen. Femoral arterial and venous catheters were inserted under direct vision. After application of a thin film of 2% lidocaine jelly to the wound edges, the animal was partially restrained in a plaster cast extending from the ankles to the lower thorax and taped to a lead block. Two hours were allowed for the animals to eliminate the halothane, during which time body temperatures were monitored with a rectal thermometer and maintained at 36–38°C with a heating lamp. Before each study, we evaluated the general health of each animal by visual inspection and with measurements of arterial blood gas tensions and mean arterial pressure to ensure that measurements of these variables were within the normal limits for our laboratory (arterial oxygen tension > 75 mmHg, arterial carbon dioxide tension 30–45 mmHg, pH 7.30–7.45, and mean arterial pressure 85–120 mmHg).

Thiopental was administered with a computer-controlled infusion pump to provide clamping at a plasma target concentration of 10 μg/ml, which has been shown to be associated with a reduction of the SMRT by approximately 30%.

Measurement of Somatic Motor Response Threshold

A modification of the paw-pressure test was used to measure the SMRT to tail pressure. Enhanced nociceptive transmission results in a decrease in the SMRT, while analgesia increases the SMRT. An Analgesy-Meter (Ugo Basile, Milan, Italy) applied a linearly increasing pressure to randomly selected points within the distal 2 cm of the tail, which was supported on a plinth. The end-point (SMRT) was defined as the first movement of the tail that could displace it from the plinth.

The behavioral status of each animal was recorded at the outset of the experiment and just before blood sampling for plasma thiopental concentrations. The behavior status was classified as awake (actively exploring the environment), drowsy (not actively exploring, but easily stimulated by manipulating the head or vibrissa), very drowsy (responds only to vigorous head manipulation), and unresponsive.

The timing of the SMRT measurements and the deoxyglucose injection were based on the program predictions of plasma thiopental levels. Control measurements of the SMRT were made at the beginning of the experiment (initial measurement). The bolus and infusion strategy were begun. Predicted steady-state plasma thiopental concentrations were achieved approximately 5 min later. The SMRT measurements were made when steady-state conditions for plasma thiopental concentrations were predicted by the computer and 3 and 6 min later (three “before 2-deoxyglucose (2-DG) measurements” in each animal). Deoxyglucose was injected 8 min after steady-state thiopental concentrations were predicted. The SMRT measurements were repeated every 2 min during the first 14 min of the 2-DG experiment (seven “during 2-DG measurements” in each animal). Blood samples for plasma thiopental concentration were drawn 2 min (“before 2-DG” value) and 10 min (“during 2-DG” value) after the computer predicted a steady-state plasma thiopental concentration.

Thiopental concentrations in plasma were measured by high performance liquid chromatography with spectrophotometric detection as previously described. The total volume of blood samples withdrawn from each animal was approximately 2 ml (15 × 100 μl (2-DG study) and 2 × 250 μl for thiopental measurements). Blood samples were replaced with a volume of normal saline equal to three times the blood sample volume.

Measurement of Local Cerebral Glucose Utilization

LCGU was measured with the autoradiographic 2-DG method of Sokoloff et al. The experiment began with the injection of 30 μCi of [14C]DG over 30 s (2-deoxy-D-glucose-specific activity of 35–40 mCi/mmol, New England Nuclear, Boston, MA). 2-DG was injected 8 min after the STANPUMP program had predicted a...
steady-state plasma thiopental concentration. Control animals were treated identically to thiopental-treated animals; the syringe contained saline. Timed arterial sampling for plasma concentrations of [14C]DG and glucose began 15 s after the start of the injection and continued for 45 min. Plasma glucose concentration was measured with a Beckman Glucose Analyzer2 (Beckman Instruments, Brea, CA). Plasma [14C]DG content was measured with a liquid scintillation counter (model 1219, LKB Rackbeta Liquid Scintillation Counter, Allied Scientific, Montreal, Canada) with calibrated [14C] toluidine internal standards (New England Nuclear). Animals were killed by decapitation and the brains rapidly removed and frozen in isopentane cooled to −50°C to −60°C by liquid nitrogen vapor. Brains were cut into 20-μm-thick sections in a cryostat (American Optical, Buffalo, NY). Autoradiographs were prepared from the dried brain sections, using calibrated [14C] methylmethacrylate (New England Nuclear) as internal standards for each autoradiograph. The autoradiographic images were analyzed with a microcomputer imaging device (MCID, Imaging Research, St. Catharines, Ontario, Canada).

Data Analysis: Local Cerebral Glucose Utilization

Before analysis, regions of interest (ROIs) were defined and identified in a rat stereotactic atlas20 and used to construct a template for the image analyzer. To test our hypothesis, 19 specific ROIs were defined. On the basis of recent human21,22 and rat studies23 forebrain regions representing nociception were chosen in the ventroposterolateral nucleus of the thalamus, in the cingulate gyrus (area 29c, Von Economo classification24), and the primary and secondary (association) somatosensory cortices for the hind limb. Brainstem regions selected were those thought to be involved in the bulbospinal endogenous pain modulating system (amygdala periaqueductal gray, nucleus accumbens, nucleus raphe magnus, and nucleus gigantocellularis).24-26 The habenula was included as part of the mesolimbic antinociceptive loop.27 For comparison, we selected structures in the visual and auditory systems.

The LCGU values within each ROI were analyzed to determine whether there was any relative change in metabolic activity during hyperalgesia with thiopental. The analysis follows the procedures commonly used for functional brain mapping with positron emission tomography.21,25 To evaluate the relative changes in the metabolic activity associated with thio-
EFFECTS OF STIMULATION ON LCGU DURING SEDATION

to the first failure to respond to tail-clamp with either
pentobarbital (n = 5) or isoflurane (n = 3). The pattern
of LCGU in each of these five groups was charac-
terized by calculating a Z value for each of 26 brain
regions, where

\[ Z = \frac{[\text{LCGU ROI} - \text{mean global LCGU}]}{\text{standard deviation for LCGU in all regions}} \]

Many of the brain regions responsible for nociceptive
processing were not evaluated in the previous study. For
pattern analysis, the regions selected were: (1) cortical
regions: visual, auditory, parietal, sensory/motor, and
frontal; (2) subcortical regions: superior colliculus,
 mammillary body, medial geniculate, dentate gyrus,
anterior hippocampus, lateral geniculate, amygdala,
lateral thalamus, ventral thalamus, habenula, hypothala-
mus, globus pallidus, and caudate; and (3) brainstem
regions: cerebellar cortex, cochlear nucleus, vestibular
nucleus, superior olive, lateral lemniscus, pontine gray,
and inferior colliculus. Z-score pattern analysis usually
is followed by Q-component factor analysis to assign
statistical significance to differences in the patterns. This
was not possible with the small number of subjects
in the current studies, so the significance of the patterns
calculated remains unresolved.

Results

The effects of noxious stimulation and thiopental
infusion on the measured physiologic variables are sum-
marized in Table 1. There were no significant differences
between initial values determined in the control and
thiopental-treated animals. In the control group, the
final mean \( P_{aCO_2} \) value was significantly less \( 37 \pm 2 \) mmHg; \( P < 0.05 \) than the initial values \( 41 \pm 2 \) mmHg) and the final blood glucose value \( 8.3 \pm 0.94 \) mmol/dl significantly greater than initial values \( 6.9 \pm 0.94 \) mmol/dl. Thiopental infusion caused a respira-
tory acidosis: the \( P_{aCO_2} \) increased from \( 40 \pm 2 \) to \( 44 \pm 4 \) mmHg \( P < 0.05 \), and the \( pH \) decreased from \( 7.43 \pm 0.01 \) to \( 7.35 \pm 0.02 \) \( P < 0.05 \). Rectal temperature fell in the thiopental treated animals, from \( 36.7 \pm 0.7^\circ C \) to \( 35.4 \pm 1.2^\circ C \) \( P < 0.05 \).

The infusion strategy provided plasma thiopental
concentrations within the target range of 10–15 \( \mu g/\)
ml throughout the period of noxious stimulation and
nociceptive threshold measurements. Plasma thiopental
concentrations before and during the 2-DG study
were \( 11.1 \pm 1.8 \) and \( 13.0 \pm 2.1 \) \( \mu g/ml \), respectively
(table 2). The initial SMRT values were similar in the
control and thiopental-treated groups. The thiopental
infusion decreased the SMRT from \( 277 \pm 32 \) to \( 215 \pm
25 \) g before the 2-DG injection, and this reduction was
maintained throughout the first 20 min of the 2-DG
experiment \( 215 \pm 41 \) g, \( P < 0.001 \). The SMRT did
not change in the control group \( P = 0.68 \). All of the
animals in the thiopental group showed a behavioral
effect from the thiopental. Three animals were classified
as “drowsy” and four were “very drowsy.”

The results of the 2-DG measurements are summa-
rized in Table 3. The global mean LCGU in thiopental-
treated animals \( (60 \pm 9 \mu mol \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}) \) was
significantly less than in the control group \( (85 \pm 5
\mu mol \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}), P < 0.001 \). The regional
differences in normalized LCGU were confirmed to be
normally distributed by the Kolmogorov-Smirnov test
(K-S distance = 0.06, \( P = 0.052 \)). Analysis of the
\( \Delta \text{LCGU} \) values by analysis of variance revealed that

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Table 1. Physiologic Values

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 7)</th>
<th>Final</th>
<th>Thiopental Treated (n = 7)</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P_{aCO_2} ) (mmHg)</td>
<td>41 ± 2</td>
<td>37 ± 2*</td>
<td>40 ± 2</td>
<td>48 ± 4*</td>
</tr>
<tr>
<td>( P_{aO_2} ) (mmHg)</td>
<td>85 ± 7</td>
<td>98 ± 10</td>
<td>92 ± 7</td>
<td>98 ± 5</td>
</tr>
<tr>
<td>( pH )</td>
<td>7.43 ± 0.01</td>
<td>7.43 ± 0.01</td>
<td>7.43 ± 0.01</td>
<td>7.35 ± 0.02*</td>
</tr>
<tr>
<td>Rectal temperature (°C)</td>
<td>36.5 ± 0.5</td>
<td>36.6 ± 0.4</td>
<td>36.7 ± 0.7</td>
<td>35.4 ± 1.2*</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>127 ± 8</td>
<td>127 ± 16</td>
<td>128 ± 4</td>
<td>127 ± 17</td>
</tr>
<tr>
<td>[Glucose] (mmol·dl⁻¹)</td>
<td>6.8 ± 0.94</td>
<td>8.3 ± 0.95*</td>
<td>7.6 ± 1.0</td>
<td>7.4 ± 0.5</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>335 ± 22</td>
<td></td>
<td>316 ± 20</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD for 7 animals. Animals were weighed before catheter insertions.
* \( P < 0.05 \) by ANOVA versus initial values within the group.
\( P_{aCO_2}, P_{aO_2} \) = arterial tensions of carbon dioxide and oxygen, respectively; [Glucose] = plasma glucose concentration; initial and final = sampling times at the beginning and end, respectively, of the 2-DG experiment.

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Table 2. Somatic Motor Response Threshold Values

<table>
<thead>
<tr>
<th></th>
<th>Initial Measurement</th>
<th>Before 2-DG Measurement</th>
<th>During 2-DG Measurement</th>
<th>Within Group Analysis (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMRT control group (7 animals)</td>
<td>247 ± 26</td>
<td>237 ± 33</td>
<td>235 ± 34</td>
<td>F = 0.386, P = 0.68</td>
</tr>
<tr>
<td>SMRT thiopeptinal treated group (7 animals)</td>
<td>277 ± 32</td>
<td>215 ± 25*</td>
<td>215 ± 41*</td>
<td>F = 8.21, P = 0.003</td>
</tr>
<tr>
<td>Plasma thiopeptinal concentration (µg·ml⁻¹)</td>
<td>11.1 ± 1.8</td>
<td>13.0 ± 2.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD. SMRT = measurements of the somatic motor response threshold.
* Significant (P < 0.05) difference from the initial measurement.

...there were significant differences among the regions (F = 6.043, P < 0.001). The state-dependent changes in LC activity in cortical regions postulated to be involved in nociception (primary somatosensory cortex, secondary somatosensory cortex, and cingulate cortex; +3.4 ± 14%) were significantly different from the visual and auditory cortex (-18 ± 15%, P = 0.012). Although there were significant changes in two structures in the mesolimbic antinociceptive loop, the nucleus accumbens and the habenular nucleus, the structures involved in the descending pain modulating system (periaqueductal gray and nucleus raphe magnus) did not show significant state-dependent differences.

Figure 1 shows the pattern of LC activity in 26 brain regions. The value Z quantifies, in units of standard deviation, the difference between the mean LC activity for the structure and the global mean LC activity across all structures. Thus, in this analysis, none of the structures in the cortical or brainstem regions was more than approximately 1.5 standard deviation units away from the global mean in any of the treatment groups. In the cortical and subcortical regions, there appear to be two consistent patterns. The animals subjected to tail pressure, with or without a thiopeptinal infusion, demonstrate a pattern of regional LC activity similar to unstimulated normal control rats. The animals at surgical levels of anesthesia with pentobarbital or isoflurane show patterns in which LC activity appears to be less than in the unanesthetized groups. The brainstem structures showed much greater variability in all groups studied, and we cannot discern any simple patterns in these structures.

Discussion

The main findings of this study were that, during a thiopeptinal infusion that decreased the SMRT to tail pressure by 23% (P < 0.001), global LC activity decreased by 29% (P < 0.001), and LC activity in cortical regions postulated to be involved in nociceptive processing was increased relative to the other cortical regions studied (P = 0.012). Thus the results support the hypothesis that cortical processing of noxious stimuli was increased (relative to other cortical sensory regions) during thiopeptinal infusions that reduce the SMRT. However, this may have been the result of enhanced nociceptive processing rather than the cause. Our initial hope was that the results would demonstrate a consistent pattern in LC activity throughout the cell assemblies that are hypothesized to be involved in pain perception. Specifically, we anticipated that the study would show increased LC activity in sensory regions involved in both the affective and the discriminative aspects of pain perception, coupled with depression of LC activity in the brainstem nuclei involved in the descending pain modulating system. Such findings would be consistent with current hypotheses concerning the mechanisms of barbiturate-induced hyperalgesia. However, the results of the current study do not conform to such a pattern and fail to support selective depression of the periaqueductal gray and the nucleus raphe magnus as a mechanism for the reduction in the SMRT observed.

LC activity during the thiopeptinal infusion was significantly different in two of the mesolimbic nuclei involved in antinociception, the nucleus accumbens (+17%) and the habenular nucleus (-17%), and in structures in the auditory system. The relative increase in the nucleus accumbens is not likely to be responsible for the increase in the SMRT because activation of this nucleus is thought to have antinociceptive effects. The likely effect of a relative depression of the habenula on nociception is not clear to us. The pattern of changes within the auditory system was not consistent. The auditory cortex and medial geniculate were relatively depres...
Effects of Stimulation on LCGU During Sedation

Table 3. Local Cerebral Glucose Utilization in 19 Regions of Interest

<table>
<thead>
<tr>
<th>Region</th>
<th>Control (n = 7)</th>
<th>Thiopental (n = 7)</th>
<th>Normalized LCGU (%)</th>
<th>ΔLCGU (%) (CI)</th>
<th>t-Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forebrain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>84 ± 9</td>
<td>64 ± 9</td>
<td>98 ± 9</td>
<td>106 ± 6</td>
<td>8 (7)</td>
</tr>
<tr>
<td>Cingulate cortex</td>
<td>101 ± 10</td>
<td>73 ± 14</td>
<td>118 ± 9</td>
<td>120 ± 14</td>
<td>3 (18)</td>
</tr>
<tr>
<td>S2</td>
<td>92 ± 9</td>
<td>65 ± 11</td>
<td>108 ± 9</td>
<td>107 ± 5</td>
<td>0 (11)</td>
</tr>
<tr>
<td>Thalamus, VPL nucleus</td>
<td>81 ± 8</td>
<td>53 ± 8</td>
<td>95 ± 11</td>
<td>88 ± 5</td>
<td>-7 (9)</td>
</tr>
<tr>
<td>Brainstem nuclei</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periaqueductal gray</td>
<td>65 ± 6</td>
<td>42 ± 8</td>
<td>76 ± 9</td>
<td>70 ± 11</td>
<td>-6 (12)</td>
</tr>
<tr>
<td>Nucleus accumens</td>
<td>67 ± 8</td>
<td>57 ± 10</td>
<td>78 ± 6</td>
<td>95 ± 15</td>
<td>17 (15)</td>
</tr>
<tr>
<td>Nucleus raphe magnus</td>
<td>57 ± 7</td>
<td>40 ± 11</td>
<td>67 ± 7</td>
<td>67 ± 15</td>
<td>0 (11)</td>
</tr>
<tr>
<td>Nucleus gigantocellularis</td>
<td>47 ± 5</td>
<td>40 ± 6</td>
<td>55 ± 6</td>
<td>65 ± 5</td>
<td>10 (8)</td>
</tr>
<tr>
<td>Amygdala</td>
<td>48 ± 6</td>
<td>40 ± 9</td>
<td>56 ± 6</td>
<td>65 ± 8</td>
<td>9 (10)</td>
</tr>
<tr>
<td>Habenula</td>
<td>101 ± 11</td>
<td>61 ± 9</td>
<td>118 ± 9</td>
<td>101 ± 7</td>
<td>-17 (5)</td>
</tr>
<tr>
<td>Visual system</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visual cortex</td>
<td>98 ± 8</td>
<td>61 ± 8</td>
<td>114 ± 7</td>
<td>100 ± 8</td>
<td>-14 (13)</td>
</tr>
<tr>
<td>Lateral geniculate body</td>
<td>76 ± 8</td>
<td>49 ± 8</td>
<td>89 ± 6</td>
<td>80 ± 7</td>
<td>-8 (10)</td>
</tr>
<tr>
<td>Superior colliculus</td>
<td>80 ± 8</td>
<td>47 ± 10</td>
<td>93 ± 6</td>
<td>78 ± 16</td>
<td>-15 (11)</td>
</tr>
<tr>
<td>Auditory system</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Auditory cortex</td>
<td>133 ± 2</td>
<td>82 ± 16</td>
<td>156 ± 7</td>
<td>134 ± 9</td>
<td>-22 (10)</td>
</tr>
<tr>
<td>Medial geniculate body</td>
<td>109 ± 3</td>
<td>68 ± 14</td>
<td>127 ± 7</td>
<td>111 ± 10</td>
<td>-16 (9)</td>
</tr>
<tr>
<td>Inferior colliculus</td>
<td>152 ± 12</td>
<td>103 ± 15</td>
<td>178 ± 11</td>
<td>171 ± 22</td>
<td>-7 (28)</td>
</tr>
<tr>
<td>Lateral lemniscus</td>
<td>109 ± 9</td>
<td>93 ± 14</td>
<td>128 ± 7</td>
<td>154 ± 13</td>
<td>26 (15)</td>
</tr>
<tr>
<td>Superior olive</td>
<td>123 ± 13</td>
<td>110 ± 18</td>
<td>143 ± 13</td>
<td>182 ± 24</td>
<td>08 (29)</td>
</tr>
<tr>
<td>Cochlear nucleus</td>
<td>102 ± 13</td>
<td>79 ± 16</td>
<td>119 ± 10</td>
<td>130 ± 18</td>
<td>10 (18)</td>
</tr>
</tbody>
</table>

LCGU values are µmol·100 g⁻¹·min⁻¹, mean ± SD, n represents the number of animals. Normalized LCGU (%) = [LCGU in each region/global LCGU for each animal] × 100; ΔLCGU = state-dependent difference in normalized LCGU values between the thiopental-treated and the control animals; CI = 95% confidence interval for the mean difference; S1 and S2 = primary and secondary somatosensory cortices, respectively.

* P < 0.05.

Compressed, and the lateral lemniscus and the superior olive showed a relative increase in activity. These data suggest that the functional effects of hyperalgesic doses of thiopental were heterogeneous within the auditory and subcortical systems subserving nociception.

The SMRT to tail pressure was used to quantify the response to the noxious stimulus in this study because this measure of nociception can be measured easily under the conditions required for autoradiography and because our laboratory previously determined the effects of thiopental on the SMRT. Rampil presented evidence to show that descending cortical and bulbar systems do not influence the concentration of isoflurane required to abolish the SMRT. This result was obtained at much higher anesthetic concentrations than those used in the current study. For example, in our model of thiopental infusion, unresponsiveness to head stimulation usually occurs at plasma thiopental concentrations greater than 25–30 µg/ml, and the animals in this state are still responsive to tail-clamp. When administering barbiturates or isoflurane, anesthetic depth has a major influence on LCGU throughout the brain.

Surgical anesthesia with pentobarbital or isoflurane was associated with a 50% decrease in global LCGU in comparison to the 29% decrease observed in the current study with thiopental. The Z value patterns presented in figure 1 suggest that surgical anesthesia with either pentobarbital or isoflurane is associated with a reduction in LCGU variability, probably due to the large decrease in glucose metabolism throughout the brain. In contrast, the pattern for thiopental remains similar to that for the unstimulated awake controls. Cleland et al. suggest that supraspinal mechanisms are important in barbiturate-induced enhancement of nociceptive transmission, including effects on the SMRT. Thus we believe that, although the SMRT may be a spinal cord reflex, it may be a useful marker to identify states of enhanced nociceptive transmission. In the current study, we have used functional brain mapping with LCGU to simultaneously examine many of the brain
regions involved in nociception, LCGR has been used previously to identify focal activation of brain regions by physiologic and experimental stimuli. During stimulation of peripheral nerves, LCGR increased above the afferent synaptic terminals, not in the cell bodies of the dorsal root ganglion. The investigators proposed that, at the cellular level, the increase in LCGR was related to increased activity of synaptic Na⁺/K⁺-ATPase required to restore ionic gradients after each action potential. If this result applies generally, then an increase in LCGR in a structure means that input to that structure is increased, without specifying whether that input is excitatory or inhibitory. In the current study this would imply, for example, that the increase in LCGR in the nucleus accumbens (+17%) reflected an increase in afferent input to that nucleus.

The results in the current study do not support the hypothesis that the hyperalgesic effects of thiopeptol result from enhancement of processing throughout the primary somatosensory pathway. The most that can be said from this study is that cortical regions proposed to be involved in nociceptive processing were relatively activated during hyperalgesia in comparison to other cortical regions, which were relatively depressed. Because both groups of animals received noxious stimulation of an intensity adjusted to the threshold of response, this relative increase in LCGR may represent enhanced cortical nociceptive processing during hyperalgesia. However, these results also may be explained simply by a relatively greater depression of unstimulated pathways by thiopeptol, with retained signal transmission and activation of the cortical projection of the primary somatosensory cortex.

The physiologic changes caused by the thiopeptol infusion (decrease in rectal temperature and respiratory acidosis) were expected, because it was not practical to either servocontrol body temperature or to ventilate the lungs of sedated animals. Because the temperature coefficient Q₁₀ between 37°C and 27°C is 2.2–2.4, the temperature drop of 1.2°C likely decreased global LCGR by approximately 8%. Although the increase in Pa₂CO₂ from 40 ± 2 to 48 ± 4 mmHg in the thiopeptol group would be likely to increase cerebral blood flow, we are not aware of any evidence that changes of this magnitude would influence LCGR. The effects of these physiologic changes on LCGR would influence global LCGR and, therefore, would have been minimized by the normalization procedures. We are not aware of any evidence that focal changes in LCGR result from changes in body temperature or Pa₂CO₂ in this range.

It is difficult to assess the risk of a type II error in this study, because it is not clear what difference in LCGR we should expect and it is difficult to determine the power of the study to detect a pattern of LCGR change among several brain regions. The study is too small to detect isolated cortical LCGR increases of 9–11% associated with unilateral femoral nerve stimulation (for a t test with a difference of 10%, SD 14%, α = 0.05, β = 0.80, then n = 32 in each of the two groups). In contrast, in a study of chronic femoral nerve constriction, cortical LCGR increased 50–100%. The current study has adequate power to detect isolated LCGR changes of 25% (for an unpaired t test with seven subjects in each group, SD ΔLCGR = 14%, α = 0.05, β = 0.813).

This study design has several important limitations that may be responsible for the failure to demonstrate specific enhancement of cell assemblies responsible for forebrain nociceptive processing. For cortical regions, we did not establish the exact location of the primary somatosensory cortex for the tail with an innocuous stimulus, such as vibration. Although we used the standard atlas locations, the activation may be sufficient focal that this method could have missed an activation that was present. This limitation does not apply to the anatomic locations of the subcortical and brainstem nuclei, which were identified easily on the

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Processing during experiments also may be affected by depression of the hypothalamic system, with retained sympathetic activity of the cortical projection pathway. Autoradiographs. Despite our efforts to minimize extraneous stimulation during the 45 min of the LCGU measurement, the rats may have received unintentional visual, auditory, and tactile stimuli. Although the control animals were treated in the same fashion, this nonspecific stimulation may have contributed to variance in the measurements that made isolation of focal activation more difficult. We chose to stimulate the tail because this can be done without moving or disturbing the animal. Fore-paw or hind-paw stimulation usually requires that the animal be lightly restrained in a towel. The choice of the tail unfortunately precluded side-to-side comparison of LCGU in each animal, a technique that may have been helpful in identification of small focal regions of activation.

In summary, the results of this study suggest that the cerebral functional effects of sedative concentrations of thiopental are heterogeneous. The distribution of metabolic changes does not support the hypothesis that enhanced nociceptive transmission is due to activation of the primary nociceptive pathways or selective depression of brainstem pain modulating nuclei. The results of this study require confirmation, particularly in experiments using stimulation paradigms in awake animals that can demonstrate activation of the primary somatosensory cortex.

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