Thiopental Potentiation of Isolated Rabbit Pulmonary Artery Contractions with Alpha Receptor Agonists

Satoru Fukuda, M.D.,* Ichiro Inomata, M.D.,* Tatsuo Tsuji, D.D.S.,† Hiroshi Takeda, M.D.‡

The effects of thiopental sodium on the adrenergic neurotransmitter junction were studied in isolated rabbit pulmonary arteries. Basal tension was not altered by thiopental (2 × 10^{-3} and 10^{-4} M) but was increased by high concentrations of thiopental (5 × 10^{-4} M). Thiopental (10^{-4} and 5 × 10^{-4} M) potentiated contractions induced by transmural electrical stimulation. Contractile responses to exogenously applied low concentrations of norepinephrine (NE) were potentiated by thiopental (2 × 10^{-4}, 10^{-4} and 5 × 10^{-4} M), whereas those to high concentrations were not altered. In strips previously incubated in 1-naphtyl-3H-NE (10^{-7} M), the release of 3H induced by transmural stimulation (5 Hz) was not altered by thiopental (10^{-4} and 5 × 10^{-4} M). Potentiation by thiopental (10^{-4} M) of the responses to transmural stimulation was not affected by prior application of cocaine or hydrocortisone. Contractions induced by alpha receptor agonists (phenylephrine and methoxamine) were potentiated by thiopental (10^{-4} M), while those induced by acetylcholine were not altered. Contractile responses to potassium chloride were attenuated by thiopental (10^{-4} M). Ambobarbital sodium and pentobarbital sodium (10^{-4} M, respectively) attenuated contractions induced by NE. It may be concluded that thiopental specifically increases the responsiveness of postsynaptic alpha receptors to NE. (Key words: Anesthetics, intravenous: thiopental. Receptors: adrenergic, alpha receptor. Sympathetic nervous system: alpha receptor, catecholamine, norepinephrine.)

HEMODYNAMIC CHANGES during thiopental anesthesia are manifested by its combined effects on the central nervous system, heart, and peripheral vasculature.1–3 Among these, the effects of thiopental on the vasculature are controversial. Price and Price2 showed that thiopental increased contraction induced by norepinephrine (NE) in the rabbit aorta and suggested an increased responsiveness to sympathetic stimulation. Burn and Hobbs,5 using perfused rabbit ear arteries, suggested an indirect NE releasing action of thiopental from stores in the vessel wall. In contrast, Altura and Altura6 reported that thiopental can exert an inhibitory action on epinephrine-induced contractions in the isolated rat aorta and portal vein. Little is known concerning the precise mechanism of action of thiopental on vascular smooth muscle in relation to the sympathetic neuroeffector junction. Thus, the present study was aimed to clarify thiopental-induced alterations of the contractile responses to transmural stimulation and to several exogenously applied agonists (NE, methoxamine, phenylephrine, acetylcholine, and potassium chloride) using the isolated rabbit pulmonary artery.

Methods

Male albino rabbits weighing 1.8–2.5 kg were anesthetized with ether and killed by bleeding from the carotid arteries. Helical strips of main pulmonary arteries (15 × 2 mm) were prepared and mounted for superfusion. The strips were suspended in a moist tunnel-shaped chamber maintained at 37°C and were superfused at 1 ml/min by a constant-flow roller pump (MP-3A, Tokyo Rikakikai, Tokyo) with Krebs Ringer bicarbonate solution (pH 7.37–7.40) containing ascorbic acid (0.1 μg/ml) and EDTA (0.15 ng/ml), which was maintained at 37°C and aerated with 95% O_2 and 5% CO_2. The composition of Krebs Ringer bicarbonate solution was as follows (mM): Na^+ 143.0; K^+ 5.9; Ca^{2+} 2.5; Mg^{2+} 1.2; Cl^− 153.9; HCO_3^{-} 25.0; SO_4^{2−} 1.2; H_2PO_4^{-} 1.2; dextrose 10.0. The resting tension was adjusted to 1.5 g.

To stimulate adrenergic nerve endings in the strips, stimulating electrodes of platinum wire (0.5 mm diameter) were placed parallel to each other on both sides of the strip. The gaps between the electrode and strip were wide enough to allow for undisturbed contraction and yet sufficiently narrow to permit effective stimulation of intramural nerve terminals. The intrinsic adrenergic nerve terminals that remained in the pulmonary arterial wall were stimulated by 0.3-ms square wave pulses with supramaximal intensity (20 V) at frequencies of 2, 5, 10, and 20 Hz (SEN-3010, Nihon Kohden Kogyo, Tokyo). The effects of bretylium (2 × 10^{-6} M) or phenolamine (10^{-4} M) on the contractile responses to transmural stimulation (20 Hz for 10 s) were tested in four strips for each drug to ascertain the effective stimulation of intramural nerve terminals. In eight strips, transmural stimulation at four different frequencies was applied repeatedly until steady responses were obtained and the effects of thiopental (2 × 10^{-3}, 10^{-3}, and 5 × 10^{-4} M) on the responses to transmural stimulation were studied. The number of electrical pulses was kept constant (200 pulses) by changing the period of stimulation (100, 40, 20, and 10 s for frequencies of 2, 5, 10, and 20 Hz, respectively). In another 41 strips, the effects of thiopental (10^{-4} M) on the responses to transmural stimulation at 5 Hz for 20 s in strips pretreated with cocaine (3 × 10^{-6} and 10^{-5} M) or hydrocortisone (4 × 10^{-6} and 2 × 10^{-4} M)
also were tested. In this experiment, stimulatory condition at 5 Hz for 20 s was used, since it was found in transmural stimulation study at four different frequencies that electrical stimulation at 5 Hz for 40 s produced near 100% maximum response. Contractions with stimulation at 5 Hz for 20 s were 62.6 ± 2.8% (n = 7) of those at 5 Hz for 40 s.

For the measurement of release of NE induced by electrical stimulation, 16 strips were incubated at 37°C for 60 min with oxygenated Krebs Ringer bicarbonate solution containing 1-[7,8-3H]-NE, 10^-7 M (5 μCi, specific activity 38.6 Ci/m mole, Radiochemical Center, Amersham, United Kingdom) as described by Su and Bevan. After rinsing for 10 min with NE free Krebs Ringer bicarbonate solutions, the strips were superfused. The spontaneous efflux of [3H] activity declined exponentially and reached a low steady level within 90 min. Stimulation (5 Hz for 20 s) started after 90(S1), 120(S2), 150(S3), 180(S4), and 210(S5) min of superfusion. The superfusate was collected for 1 min, immediately before stimulation (basal efflux) and the first, second, third, and fourth minute after the start of stimulation. Fifteen milliliters of ACS II solution (Amersham/Searle Corporation, Des Plaines, Illinois) was added to each sample, and total [3H] activities expressed as counts per minute were determined using a liquid scintillation spectrometer (Packard 3330). The total [3H] overflow above basal efflux resulting from stimulation was calculated as the sum of difference between basal efflux and the efflux detected in each of four samples. As the [3H] overflow induced by initial stimulation (S1) was variable, the mean [3H] overflow induced by S2 and S3 ([S2,3]) was used as control. Thiopental (10^-4 and 5 × 10^-4 M) was added 15 min before S4. Its effects were expressed as the ratio between the [3H] overflow induced by S4 or S5 ([S4] or [S5]) and the mean overflow induced by S2 and S3 ([S2,3]), respectively.

Five strips were exposed to a solution containing thiopental (2 × 10^-5, 10^-4, and 5 × 10^-4 M) for 20 min to determine whether thiopental affected basal tension in the absence and presence of phenoxybenzamine (10^-5 M). Phenoxybenzamine was added 60 min before thiopental administration.

The effects of 2 × 10^-5, 10^-4, and 5 × 10^-4 M of thiopental on contractions induced by various concentrations of NE were tested in seven, eight, and six strips, respectively. After the tensions induced by NE stabilized, thiopental was added to the superfusion media. In another four strips, steady contractions induced by NE (8 × 10^-9 M) were obtained first, and the strips were treated with thiopental (5 × 10^-4 M) for 15 min, and then NE (8 × 10^-9 M) was added.

The effects of thiopental (10^-4 M) on contractions induced by various concentrations of phenylephrine, methoxamine, acetylcholine, and potassium chloride also were tested in six strips for each agonist. The effects of thiopental on contractions induced by potassium chloride were tested in the presence of pentobarbital (10^-6 M), since high concentrations of potassium chloride release NE from the nerve terminals remaining in the vascular wall.

The effects of other barbiturates (10^-4 M amobarbital sodium and pentobarbital sodium) on contractions induced by NE (8 × 10^-9 M) also were examined in three strips for each drug.

Two strips of pulmonary arteries of approximately the same length and diameter were isolated from the same rabbit. One served as control group (n = 6) and the other as thiopental group (n = 6). Contractions induced by NE (8 × 10^-9 M) were obtained first until steady contractions were obtained. The strips were exposed for 5 min to Ca^2+ free Krebs Ringer bicarbonate solution containing 0.1 mM EDTA (glycolate-diamine-N,N,N',N'-tetraacetic acid) and subsequently for 5 min they were treated with the Ca^2+ free EDTA media containing either Na_2CO_3 (control group) or thiopental (10^-4 M) (thiopental group). Norepinephrine (8 × 10^-9 M) was then added and 5 min later, calcium (2.5 mM) was added.

Values presented in the text and figures are mean ± SEM. The data were analyzed statistically by Student's paired or unpaired t test; P < 0.05 was considered to be significant. Drugs used were dl-norepinephrine hydrochloride (Sankyo Pharmaceutical Co.), acetylcholine chloride (Nakarai Chemical Ltd.), cocaine hydrochloride (Takeda Pharmaceutical Co.), and hydrocortisone succinate (Nikken Pharmaceutical Co.). Barbiturates used were thiopental sodium (Tanabe Pharmaceutical Co.), amobarbital sodium (Yoshitomi Pharmaceutical Co.), and pentobarbital sodium (Dainippon Seiyaku Pharmaceutical Co.). The powder of thiopental sodium contained 0.3% Na_2CO_3 for preservation. Thus, the same concentration of Na_2CO_3 as that contained in the thiopental sodium solution was used in a control experiment. It was preliminarily confirmed that Na_2CO_3 did not exert any influence on the contractions induced by transmural stimulation and exogenously applied NE.

**Results**

Contractile responses to transmural stimulation were abolished by treatment for 20 min with bretylium (2 × 10^-5 M) or pentobarbital (10^-6 M).

Treatment with thiopental (2 × 10^-5 M) did not alter the response to transmural stimulation. However, thiopental (10^-4 and 5 × 10^-4 M) significantly potentiated the responses to stimulation at 2, 5, and 10 Hz (fig. 1). Cocaine (3 × 10^-6 and 10^-5 M) and hydrocortisone (4 × 10^-5 and 2 × 10^-4 M) significantly potentiated the contractions by transmural stimulation. Maximum contractions with cocaine and hydrocortisone were obtained in
concentrations of $3 \times 10^{-6}$ and $2 \times 10^{-4}$ M, respectively. Prior application of cocaine ($3 \times 10^{-6}$ and $10^{-5}$ M) or hydrocortisone ($4 \times 10^{-6}$ and $2 \times 10^{-4}$ M) did not prevent the potentiating action of thiopental ($10^{-4}$ M) (Tables 1 and 2). Basal effluxes were not changed by thiopental ($10^{-4}$ and $5 \times 10^{-4}$ M). The ratio between $[S_4]$ or $[S_8]$ and $[S_2,3]$ in absence and presence of thiopental was unchanged as shown in Table 3.

The basal tension was not altered by thiopental ($2 \times 10^{-5}$ and $10^{-3}$ M). High concentrations of thiopental ($5 \times 10^{-4}$ M) increased the tension by $0.64 \pm 0.18$ g (n = 5). With pretreatment of phenoxybenzamine ($10^{-5}$ M), the contractions with thiopental remained unchanged.

Contractions induced by low concentrations of NE (less than $5 \times 10^{-8}$ M) were potentiated by thiopental ($2 \times 10^{-8}$, $10^{-6}$, and $5 \times 10^{-4}$ M), whereas those induced by high concentrations (greater than $10^{-6}$ M) were not altered (fig. 2). Figure 3 (middle panel) shows a representative recording of potentiation of contraction induced by NE ($8 \times 10^{-9}$ M) with thiopental. The contractions induced by NE ($8 \times 10^{-9}$ M) increased by $124 \pm 40$% with thiopental ($5 \times 10^{-4}$ M). The contractions induced by low concentrations of phentolamine or methoxamine also were potentiated by thiopental ($10^{-4}$ M), while those induced by acetylcholine and high concentrations of phentolamine and methoxamine were not affected (fig. 3, lower panel, and fig. 4). Contractions induced by potassium chloride ($20-50$ mM) were attenuated by thiopental ($10^{-4}$ M) (fig. 4). Amobarbital and pentobarbital attenuated contractions induced by NE ($8 \times 10^{-9}$ M) by $21.1 \pm 2.2$ and $25.3 \pm 6.4$, respectively.

Addition of Ca$^{2+}$ ($2.5$ mM) to the strips previously incubated with Ca$^{2+}$-free-GEDTA-media-containing NE ($8 \times 10^{-9}$ M) caused a stable contraction. Thiopental potentiated this contraction (fig. 5).

### Table 1. Effects of Pretreatment with Cocaine on the Potentiation by Thiopental of the Contractile Response to Transmural Stimulation (5 Hz).

<table>
<thead>
<tr>
<th>Drugs</th>
<th>$n$</th>
<th>Tension (g)</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>0.51 ± 0.07</td>
<td>100</td>
</tr>
<tr>
<td>Thiopental $10^{-4}$ M</td>
<td>7</td>
<td>0.60 ± 0.07*</td>
<td>120.2 ± 4.8</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>0.55 ± 0.06</td>
<td>100</td>
</tr>
<tr>
<td>Cocaine $3 \times 10^{-6}$ M</td>
<td>8</td>
<td>0.70 ± 0.09†</td>
<td>117.3 ± 5.7</td>
</tr>
<tr>
<td>Thiopental $10^{-4}$ M</td>
<td>8</td>
<td>0.82 ± 0.11††</td>
<td>118.9 ± 2.8</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>0.51 ± 0.12</td>
<td>100</td>
</tr>
<tr>
<td>Cocaine $10^{-4}$ M</td>
<td>6</td>
<td>0.69 ± 0.17§</td>
<td>120.2 ± 4.8</td>
</tr>
<tr>
<td>Thiopental $10^{-4}$ M</td>
<td>6</td>
<td>0.80 ± 0.18†††</td>
<td>118.9 ± 2.8</td>
</tr>
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</table>

Values are mean ± SEM. Significantly different from control: *$P < 0.001$, †$P < 0.01$, ††$P < 0.05$ (paired $t$ test). Significantly different from cocaine treated values: ††$P < 0.001$, †††$P < 0.01$ (paired $t$ test).

### Table 2. Effects of Pretreatment with Hydrocortisone on the Potentiation by Thiopental of the Contractile Response to Transmural Stimulation (5 Hz).

<table>
<thead>
<tr>
<th>Drugs</th>
<th>$n$</th>
<th>Tension (g)</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>0.51 ± 0.07</td>
<td>100</td>
</tr>
<tr>
<td>Thiopental $10^{-4}$ M</td>
<td>7</td>
<td>0.60 ± 0.07*</td>
<td>120.2 ± 4.8</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>0.52 ± 0.06</td>
<td>100</td>
</tr>
<tr>
<td>Hydrocortisone $4 \times 10^{-4}$ M</td>
<td>8</td>
<td>0.65 ± 0.07*</td>
<td>120.2 ± 4.8</td>
</tr>
<tr>
<td>Thiopental $10^{-4}$ M</td>
<td>8</td>
<td>0.72 ± 0.07†††</td>
<td>118.9 ± 2.8</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>0.58 ± 0.12</td>
<td>100</td>
</tr>
<tr>
<td>Hydrocortisone $2 \times 10^{-4}$ M</td>
<td>5</td>
<td>0.74 ± 0.13§</td>
<td>120.2 ± 4.8</td>
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<tr>
<td>Thiopental $10^{-4}$ M</td>
<td>5</td>
<td>0.87 ± 0.15‡‡‡</td>
<td>118.9 ± 2.8</td>
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</table>

Values are mean ± SEM. Significantly different from control: *$P < 0.001$, †$P < 0.01$ (paired $t$ test). Significantly different from hydrocortisone-treated values: †$P < 0.001$, ††$P < 0.02$ (paired $t$ test).

**Fig. 1.** Potentiation by thiopental of the contractile responses to transmural stimulation. Closed circles indicate responses in the absence of thiopental (control: $n = 8$). Open circles, triangles and squares indicate responses in the presence of thiopental, $2 \times 10^{-5}$ M ($n = 8$), $10^{-4}$ M ($n = 8$), and $5 \times 10^{-4}$ M ($n = 8$), respectively. Response at frequency of $20$ Hz in control media was taken as $100$%; mean absolute value of the tension was $0.52 \pm 0.05$ g. Thiopental ($10^{-4}$ and $5 \times 10^{-4}$ M) potentiated the contractile responses to transmural stimulation. * indicates significant difference from control (paired $t$ test).
TABLE 3. Effects of Thiopental on the [1H] Release by Transmural Stimulation

<table>
<thead>
<tr>
<th>Drug</th>
<th>n</th>
<th>[Sd]/[Ss]</th>
<th>[Sd]/[Ss]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6</td>
<td>1.00 ± 0.02</td>
<td>1.01 ± 0.03</td>
</tr>
<tr>
<td>Thiopental 10⁻⁴ M</td>
<td>5</td>
<td>1.01 ± 0.03</td>
<td>0.99 ± 0.05</td>
</tr>
<tr>
<td>Thiopental 5 × 10⁻⁴ M</td>
<td>5</td>
<td>0.97 ± 0.03</td>
<td>0.94 ± 0.04</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Statistical analysis was done by unpaired t test.

The pH of the solutions after adding thiopental (2 × 10⁻⁵, 10⁻⁴, and 5 × 10⁻⁴ M) was not changed.

Discussion

The present study revealed that thiopental increased the responsiveness of alpha receptors to norepinephrine (NE).

Potentiation of the response to transmural stimulation similar to that observed with thiopental in this study has been shown to occur due to amine uptake inhibition,⁹,¹⁰ facilitation of NE release from nerve terminals,¹¹,¹² increased responsiveness to NE,¹³ and/or catechol-O-methyltransferase (COMT) inhibition.⁹ It is well known that cocaine has an amine neuronal uptake inhibitory action⁹ and hydrocortisone has an amine extraneuronal uptake inhibitory action.¹⁰ In the present study, prior application of cocaine or hydrocortisone sufficient to produce maximum potentiation in association with an inhibition of neuronal or extraneuronal uptake of NE did not prevent the potentiating action of thiopental. This suggests that thiopental has no amine uptake inhibitory action at the sympathetic neuroeffector junction. The finding that thiopental did not alter the basal efflux and the overflows of [¹H] by transmural stimulation excludes the possibility of facilitation of NE release from nerve terminals. Increased responsiveness can be caused by membrane depolarization of vascular smooth muscle.¹⁴ Agents such as ouabain, which cause membrane depolarization due to sodium pump inhibition at the cell membrane,¹³ potentiate contractions induced by acetylcholine and potassium chloride as well as those induced by alpha agonists, which include NE, phenylephrine, and methoxamine. However, thiopental did not potentiate the contractions induced by acetylcholine and instead attenuated the contractions.
induced by potassium chloride. It appears that the potentiating action of thiopental is not related to membrane depolarization of vascular smooth muscle. The present study also showed that contractions induced by phenylephrine and methoxamine, which act directly at alpha receptors and are not degraded by COMT, were potentiated by thiopental. Although it is not known whether thiopental has COMT inhibitory action, these findings strongly suggest that the potentiating action of thiopental is specific for alpha receptors.

The finding of no influence of phenoxybenzamine on the contractions with high concentrations of thiopental suggests that thiopental’s contractions are not caused by low concentrations of NE remaining in the synaptic clefts. It appears that contractions with thiopental are induced by thiopental’s nonspecific direct action, since phenoxybenzamine in concentrations used in the present study influences contraction with NE as well as other specific agonists.

Casteels et al. reported that low concentrations of NE (less than 10^{-7} M) elicit contractions by increasing calcium influx without membrane depolarization and do not affect calcium efflux in isolated rabbit pulmonary arteries. They further suggested that intracellular Ca^{2+} is not used in contractions induced by low concentrations of NE. Thus, the finding that addition of calcium to preparations previously incubated with Ca^{2+} free-GEDTA media containing NE (8 x 10^{-9} M) caused greater contractions in thiopental-treated preparations than in control ones suggests that thiopental enhances calcium influx with the stimulation of alpha receptors at the cell membrane. However, the possibility of potentiation by thiopental of the intracellular contractile mechanism induced by NE was not excluded.

**Figure 4.** Effects of thiopental (10^{-4} M) on the contractions induced by various concentrations of phenylephrine, methoxamine, acetylcholine, and potassium chloride. Closed and open circles indicate responses in the absence (control) and presence of thiopental (10^{-4} M), respectively. Responses to phenylephrine (5 x 10^{-6} M), methoxamine (5 x 10^{-6} M), acetylcholine (5 x 10^{-5} M), and potassium chloride (40 mM) were taken as 100%; mean absolute values of the tension were 2.79 ± 0.07 g (n = 6), 2.32 ± 0.58 g (n = 6), 0.54 ± 0.14 g (n = 6), and 2.80 ± 0.25 g (n = 6), respectively. Thiopental potentiated the contractions induced by low concentrations of phenylephrine and methoxamine, whereas those by high concentrations were not altered. The contractions induced by acetylcholine were not altered, but those by potassium chloride were attenuated. * Indicates significant difference from control (paired t test).

**Figure 5.** Potentiation by thiopental (10^{-4} M) of the Ca^{2+}-induced contractions in the presence of NE (8 x 10^{-9} M). Tension induced by NE (8 x 10^{-9} M) in normal Krebs Ringer solution was taken as 100%; mean absolute values of the tension were 1.90 ± 0.06 g in control group (n = 6) and 1.93 ± 0.07 g in thiopental group (n = 6). Addition of Ca^{2+} (2.5 mM) caused greater contractions in thiopental group than in control one. * Indicates significant difference from control (paired t test). For further explanation, see text.
In this study, contractions induced by high concentration of NE were not potentiated by thiopental. Casteels et al. reported that NE (greater than 2 x 10^-7 M) caused membrane depolarization dose-dependently in rabbit pulmonary arteries. In the present study, thiopental attenuated contractions induced by potassium chloride, which are induced by membrane depolarization. Thus, it appears that thiopental inhibits the contractile mechanism related to membrane depolarization and this inhibitory action masks the potentiating action of thiopental in the presence of high concentrations of NE.

The smooth muscle cells of rabbit pulmonary artery used in the present study possess only alpha-1 receptors. Systemic vessels such as the femoral artery, splenic artery (dog), cerebral vessels (humans, guinea pig, and rat), and aorta (hamster, rabbit, guinea pig, cat, and dog) also contain solely alpha-1 adrenoceptors. Thus, our results concerning thiopental's potentiation of NE contraction in rabbit pulmonary artery can be applied to the systemic vasculature. According to Altura and Altura, barbiturates including thiopental in concentrations above 5 x 10^-5 M inhibited contractions induced by epinephrine in rat aortas. The inhibitory actions of amobarbital and pentobarbital that they reported are consistent with our data, but the effects of thiopental on contractions induced by alpha agonists differ. Ruffolo et al. reported that alpha receptors of the rat aorta, which closely resemble alpha-2 subtypes, differ from other mammalian aortas. Discrepancies between Altura's data and ours are probably due to differences in alpha receptor subtypes.

In this study, the concentrations of thiopental-producing potentiation of contractions induced by NE were above 4.8 ìg/ml (2 x 10^-5 M). It has been reported that free plasma thiopental levels of 5.9-6.3 ìg/ml produce surgical anesthesia in humans. Thus, the concentrations of thiopental used here can be encountered during the clinical use of thiopental.

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References
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