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Selective Synaptic Actions of Thiopental and Its Enantiomers

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Background: **There is conflicting evidence concerning the extent to which the intravenous general anesthetic thiopental acts by enhancing inhibitory γ-aminobutyric acid–mediated (GABAergic) synaptic transmission or by inhibiting excitatory glutamatergic transmission. Yet there are remarkably few studies on the effects of thiopental on functional synapses. In addition, the degree of stereoselectivity of thiopental acting at synapses has yet to be tested.**

Methods: **The actions of thiopental and its enantiomers on GABAergic and glutamatergic synapses were investigated using voltage clamp techniques on microisland cultures of rat hippocampal neurons, a preparation that avoids the confounding effects of complex neuronal networks.**

Results: **Racemic thiopental markedly enhanced the charge transfer at GABAergic synapses without significantly affecting the peak of the postsynaptic current. At a surgically relevant concentration (25 μM), charge transfer was increased by approximately 230%. However, even at twice this concentration there were no significant effects on glutamatergic postsynaptic currents. At GABAergic synapses, thiopental acted stereoselectively, with the S(−) enantiomer being approximately twice as effective as the R(+) enantiomer at enhancing charge transfer.**

Conclusions: **Thiopental stereoselectively enhances inhibitory GABAergic synaptic transmission in a way that reflects animal potencies, supporting the idea that this is a principal mode of action for this drug. The absence of any effect on glutamatergic synapses at surgically relevant concentrations suggests that the inhibition of these excitatory synapses is not an important factor in producing thiopental general anesthesia.**

THIOPENTAL has been one of the most widely used intravenous general anesthetics since its introduction into clinical practice in the 1930s. However, despite its importance, there have been surprisingly few *in vitro* mechanistic studies using this barbiturate. Nonetheless, it is generally agreed that the γ-aminobutyric acid type A ($GABA_A$) receptor is a major target for anesthetic barbiturates, and that the potentiation of inhibitory GABA-mediated (GABAergic) synapses is an important mode of action for these drugs.[1–4] GABAergic synapses are not only significantly potentiated at pharmacologically relevant concentrations, but the observed stereoselectivities[5–8] roughly reflect those seen in animals,[9] thus satisfying two criteria we consider to be important for establishing relevance.[10] However, it has often been suggested that the inhibition of excitatory glutamatergic synapses also plays an important role.[11,12] Recent support for this idea comes from work on glutamate receptors in dissociated neurons,[13] cultured neurons,[14,15] *in vitro* expression systems,[16–18] and brain slice preparations,[19] although some data on functional synapses contradict this view.[20] Most of this work has been performed using pentobarbital, which, at sufficiently high concentrations, inhibits both *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors in a fashion that suggests open-channel block.[13,15] However, there is considerable disagreement in the literature as to which free aqueous concentrations of pentobarbital should be used in *in vitro* experiments to correctly reflect those that are present during surgical anesthesia. (Our calculations[2] suggest a figure of 50 μM, yet published estimates extend to as high as 200 μM.[21])

Fortunately, in the case of thiopental, there are direct measurements that have established the relevant free aqueous concentrations for a variety of anesthetic end points.[22] With this uncertainty removed, a number of significant questions now remain to be answered about the mode of action of thiopental. First, are functional glutamatergic synapses as sensitive to thiopental as glutamate receptors in nonsynaptic preparations? Sensitivities may well differ because with nonsynaptic preparations, nonphysiologic agonists are almost invariably used and applied relatively slowly, whereas at synapses, the natural neurotransmitter is, of course, glutamate that is released at high concentrations in a matter of milliseconds. Second, to what extent are GABAergic synapses potentiated and glutamatergic synapses inhibited at relevant thiopental concentrations (and are these effects postsynaptic or presynaptic)? Finally, does thiopental act stereoselectively on synapses and, if so, does the degree of stereoselectivity reflect that seen in animals? Here we address these questions using a synaptic preparation (microisland cultures) that allows the study of homogeneous populations of each type of synapse (GABAergic or glutamatergic) without the confounding effects of heterogeneous neuronal networks.[23–25]

Materials and Methods

This study conforms to the United Kingdom Animals (Scientific Procedures) Act of 1986 and has been approved by the Ethical Review Committee of the Imperial

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Culturing Hippocampal Neurons

Hippocampal neurons were grown in culture using methods described previously.[23–25] Hippocampi from Sprague-Dawley rats (postnatal days 1–3; killed by decapitation) were dissected, roughly sliced, and agitated in a papain-containing solution (20 units/ml) for 30 min at 37°C. After washing with enzyme-free solution, the tissue was gently triturated with a fire-polished Pasteur pipette, and the cells were plated out at a density of 8–10 $\times$ 10^4 cells/ml and cultured (95% air–5% CO_2) at 37°C. Glass coverslips used for culturing the cells were first coated with agarose (0.15% wt/vol) and then sprayed with a fine mist of poly-D-lysine and collagen (0.1 mg/ml poly-D-lysine and 0.5 mg/ml rat-tail collagen) from a glass microatomizer and sterilized by ultraviolet exposure. This produced microislands of permissive substrate with diameters between 100 and 1,000 μm. At 3–4 days after plating, when the glial cell layer was approximately 80% confluent, an antimitotic agent (cytosine β-D-arabinofuranoside, 5 μM) was added to arrest glial cell proliferation. Neuronal cultures were then allowed to mature for another 4–9 days. We used microislands that contained single isolated neurons whose axonal processes and dendritic trees formed multiple self-synapses (autapses). Necessarily, inhibitory synapses are only made to inhibitory neurons, whereas excitatory synapses are only made to excitatory neurons. As previously described,[23,24,26] the control synaptic currents fell into two clear populations: GABAergic and glutamatergic. These currents were, respectively, blocked by 10 μM bicuculline (94 $\pm$ 1% inhibition; n = 8) or 1 mM kynurenic acid (80 $\pm$ 3% inhibition, n = 7).

Electrophysiology

The neurons were voltage clamped using the whole cell recording technique (Axopatch 200 amplifier; Axon Instruments, Foster City, CA). Electrodes were fabricated from borosilicate glass and typically had resistances between 3 and 5 MΩ. Series resistance was compensated by 75–90%. In addition, all current traces were corrected for the effects of the uncompensated series resistance using the known linear current–voltage relations and reversal potentials.[26] Neurons were voltage clamped at $-$60 mV, and synaptic responses were stimulated by a 2-ms depolarizing pulse to +20 mV. Shortly after the restoration of the membrane potential to $-$60 mV, a large (1–20 nA) postsynaptic current was observed and recorded. For the synaptic measurements, data were sampled at 50 kHz, filtered at 20 kHz ($-$3 dB, eight-pole Bessel), and stored on a computer. The extracellular recording solution was 137 mM NaCl, 5 mM KCl, 3 mM $CaCl_2$, 5 mM HEPES, 10 mM glucose, 0.001 mM glycine, and 0.0001 mM strychnine-HCl, titrated to pH 7.3 with NaOH. (Glycine was present as it is a necessary cofactor for NMDA receptor activation, and strychnine was added to block any glycine receptors, had they been present.) The intracellular (pipette) solution was 140 mM KCl, 4 mM NaCl, 0.5 mM EGTA, 2 mM MgATP, and 10 mM HEPES, titrated to pH 7.25 with KOH.

Unless otherwise stated, all chemicals were obtained from Sigma Chemical Co. (Poole, Dorset, United Kingdom). All electrophysiologic measurements were conducted at room temperature (20–23°C).

Preparation of Thiopental Enantiomers

Milligram quantities of the enantiomers of thiopental were prepared from racemic thiopental (sodium salt, Sigma Chemical Co.) using high-performance liquid chromatography (HPLC) as described previously.[8] We used a column (250 mm long, 10 mm ID, approximately 20 ml capacity) with a chiral stationary phase consisting of permethylated β-cyclodextrin covalently bonded to silica. The column was temperature-controlled and held at 23–24°C. The mobile phase was a volatile buffer solution composed of either (1) 65% (vol/vol) methanol (HPLC Hipersolv grade; BDH Laboratory Supplies, Poole, Dorset, United Kingdom) and 35% (vol/vol) of an aqueous solution of 1.0% triethylammonium acetate (HPLC grade; Applied Biosystems, Foster City, CA) titrated to pH 4.0 with acetic acid, or (2) 60% (vol/vol) methanol and 40% (vol/vol) of 1.0% aqueous triethylammonium acetate, pH 4.0. The enantiomer separation was a two-stage process with the first purification step using buffer (1) and the secondary "clean-up" step using buffer (2) which gave better separation but had an increased retention time. After separation on the HPLC column, the enantiomers were dried on a rotary evaporator followed by a centrifugal evaporator. After the second column purification, the optical purity of the enantiomers was greater than 99.0%.

Anesthetic Solutions

Solutions of thiopental in extracellular recording solution were prepared from concentrated stock solutions made up in NaOH, and were retitrated to pH 7.3 with HCl where necessary. A maximum concentration of 50 μM thiopental was used because at higher concentrations we observed significant changes in the baseline current after drug application. Thiopental solutions were preapplied to the cells for at least 45 s before recording.

Integration of the Synaptic Responses

The decay phase of the synaptic current, I(t) (where t is the time measured from the peak of the current), was fit by a biexponential equation of the following form:

$$I(t) = I_{fast}e^{-t/\tau_{fast}} + I_{slow}e^{-t/\tau_{slow}} \quad (1)$$

where I_{fast} and I_{slow} are the amplitudes and τ_{fast} and τ_{slow} are the time constants of the fast and slow components,

Table 1. Control Parameters for Synaptic Currents

Parameter	Excitatory Currents	n*	Inhibitory Currents	n*
Time to peak (ms)	4.6 ± 0.2	42	5.4 ± 0.1	63
Decay half-time (ms)	8.9 ± 0.5	42	46.3 ± 1.4	63
τ_{fast} (ms)	9.1 ± 0.4	42	41.3 ± 1.5	63
τ_{slow} (ms)	235 ± 11	42	188 ± 7	63
I_{fast}/I_{total}†	0.85 ± 0.01	42	0.61 ± 0.01	63
Q_{slow}/Q_{total}‡	0.77 ± 0.02	42	0.70 ± 0.01	63

* number of cells; † $I_{total} = I_{fast} + I_{slow}$; ‡ $Q_{total} = Q_{fast} + Q_{slow}$.

respectively. To obtain an estimate for the total charge transfer, the excitatory postsynaptic currents (EPSCs) or inhibitory postsynaptic currents (IPSCs) were numerically integrated. However, because in some cases the currents had not decayed to baseline by the end of the recording period, a correction (which was invariably < 5% of the total charge transfer) was applied by extrapolating the observed current to the baseline using the biexponential fit to the decay phase of the response. The percentage change in a parameter, P, was calculated as follows:

$$\% \text{ effect on a parameter P} \equiv \left(\frac{P_{thiopental} - P_{control}}{P_{control}}\right) \times 100\% \quad (2)$$

where $P_{thiopental}$ is the value of parameter P in the presence of thiopental and $P_{control}$ is the value of the parameter in the absence of thiopental.

Statistical Analysis

Values throughout are given as mean ± SEM, and statistical significance was assessed with use of the Student *t* test.

Results

Control Synaptic Currents

The control parameters characterizing the GABAergic and glutamatergic currents are given in table 1 and are essentially identical to those we found in our previous study[26] on the effects of isoflurane and xenon. The much slower GABAergic currents (decay half-time, ~46 ms) were immediately distinguishable from the glutamatergic currents (decay half-time, ~9 ms). For the excitatory glutamatergic currents, approximately 23% of the charge was carried by a slow component (table 1), which has previously been identified[23,24,26] as being mediated by the NMDA-receptor subtype of the glutamate receptor superfamily.

Effects of Racemic Thiopental on Excitatory Currents

Racemic thiopental had no significant effect on excitatory glutamatergic synapses (fig. 1). Even at 50 μM, the highest concentration we tested, the effects were negli-

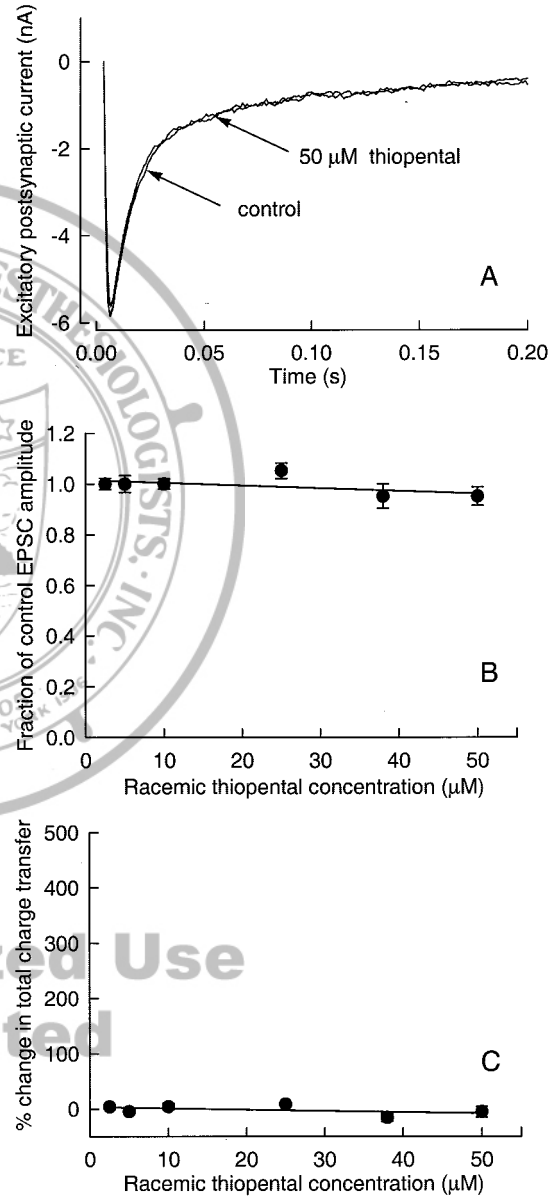

Fig. 1. Lack of effect of racemic thiopental on excitatory glutamatergic currents. (*A*) Representative traces showing the lack of effect of 50 μM thiopental on glutamatergic synaptic currents. (*B*) The amplitude of the excitatory postsynaptic current (EPSC) does not change significantly over the clinically relevant range of concentrations. (*C*) The total charge transfer (the total area under the EPSC), which also does not change significantly, is plotted as a percentage change from the control value on the same scale as that used in fig. 3C. The points represent mean values (for an average of 19 cells). In both cases the lines are drawn by eye and have no theoretical significance.

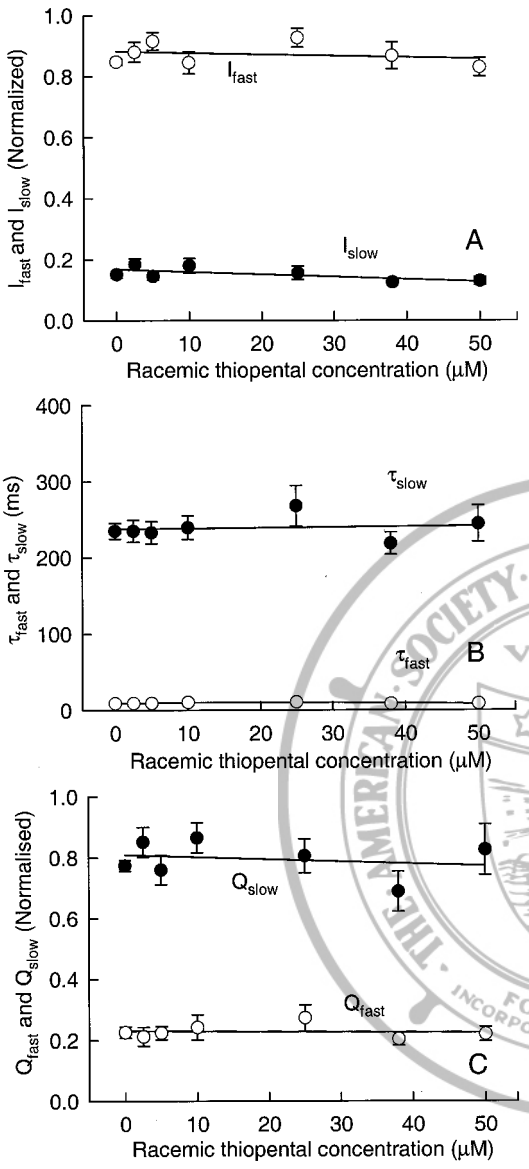

Fig. 2. The lack of effect of racemic thiopental on the fast and slow components of the excitatory postsynaptic currents derived from biexponential fits. (*A*) Amplitudes of the fast (open circles) and slow (filled circles) components, normalized such that the sum of the fast and slow components is equal to unity in the absence of anesthetic. (*B*) Fast (open circles) and slow (filled circles) time constants. (*C*) Charge transfer for the fast (open circles) and slow (filled circles) components, normalized such that the total charge transfer for the control equals unity. The points represent mean values (for an average of 22 cells). In all cases the lines are drawn by eye and have no theoretical significance.

gible. Figure 1A shows representative current traces and figures 1B and C show that, over the range of concentrations investigated, there were no effects on either the peak EPSC amplitude or the total charge transfer. The biexponential fits gave parameters for the fast and slow components of the response (fig. 2), which also showed no significant changes from control values. Table 2 gives the percentage changes in the various synaptic parameters at a concentration of 50 μM for racemic thiopental.

Effects of Racemic Thiopental on Inhibitory Currents

We next looked at the effects of racemic thiopental on inhibitory currents. We found that, even at the lowest concentration tested (2.5 μM), there was a significant prolongation of the IPSC that increased dose-dependently (fig. 3). In contrast to the large effect on the total charge transfer (fig. 3C), the peak amplitude of the IPSC was little affected (fig. 3B). At 38 μM thiopental, the total charge transfer was increased by nearly 400%, whereas the peak current was reduced by only approximately 9%, but this was not significantly different from the control amplitude (figs. 3B and C). Table 3 gives the percent changes in the various synaptic parameters at a free aqueous concentration (25 μM) that has been calculated[2] to prevent response to a painful stimulus in the rat.[22] At this concentration, the total charge transfer is increased by more than 200% (more than threefold), whereas the amplitude of the response is barely affected (~10% inhibition).

When the fast and slow components of the IPSCs were calculated from the biexponential fits (see Materials and Methods), it is clear (fig. 4) that the large increase in charge transfer is entirely the result of an increase in the charge carried by the slow component of the IPSC. However, although the charge transfer mediated by the fast component does not change significantly, this is the result of a substantial reduction in the amplitude of the fast component of the IPSC together with a compensatory increase in the time constant for this fast component (table 3).

Effects of Thiopental Enantiomers on Inhibitory Currents

When the pure thiopental enantiomers were used, the S(−) isomer was invariably found to be more effective than the R(+) isomer at prolonging the postsynaptic current. Figure 5A shows representative traces at a concentration of 10 μM, a concentration that causes a loss of righting reflex in the rat.[22] In this example, the more potent enantiomer is approximately 2.4 times more effective than the less potent enantiomer at prolonging the

Table 2. Effects of 50 μM Racemic Thiopental on Excitatory Synaptic Currents

Parameter	% change (mean ± SEM)	↑, ↓ or NS*	n†
I_{peak}	−4.9 ± 3.6	NS	19
Decay half-time	−5.2 ± 2.6	NS	19
Total charge transfer	−5.3 ± 9.4	NS	19
I_{fast}	−4.4 ± 3.2	NS	19
I_{slow}	4.4 ± 7.2	NS	19
τ_{fast}	−3.1 ± 2.0	NS	19
τ_{slow}	10.4 ± 10.2	NS	19
Q_{fast}	−2.6 ± 11.1	NS	19
Q_{slow}	6.7 ± 10.9	NS	19

* NS = not significant at the 95% confidence level (Student *t* test); † n = number of cells.

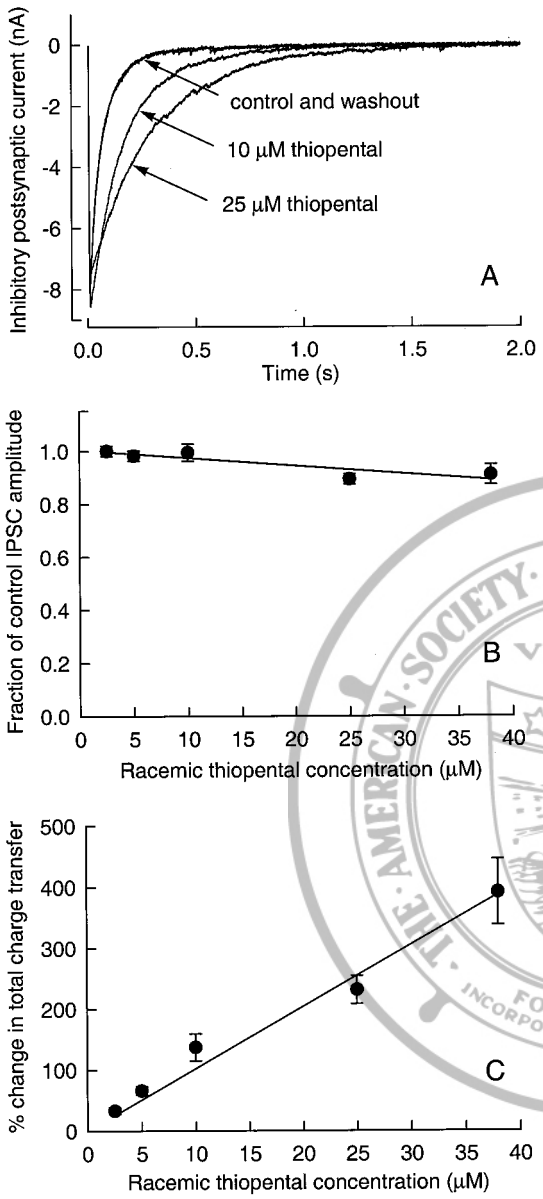

Fig. 3. The effects of racemic thiopental on inhibitory γ-aminobutyric acid–mediated (GABAergic) currents. (*A*) Representative traces showing the effects of 10 and 25 μM thiopental on GABAergic synaptic currents in the same neuron. The principal effect is a prolongation in the current decay (in this example, the decay half-time is increased by approximately 120 and 310%, respectively), with little or no effect on peak height. (*B*) The amplitude of the inhibitory postsynaptic current (IPSC) decreases monotonically with increasing concentrations of thiopental, but the effect is small. (*C*) The increase in total charge transfer (the total area under the IPSC) is plotted as a percentage change from the control value. The points represent mean values (for an average of 13 cells). In both cases the lines are drawn by eye and have no theoretical significance.

current. As with the racemic mixture, the two enantiomers had little or no effect on the peak amplitude of the IPSC (fig. 5B) but substantial effects on the total charge transfer (fig. 5C). Although both enantiomers markedly increased the charge transfer, the S(−) isomer was between approximately 1.5 and 2.5 times more effective than the R(+) isomer over the pharmacologically relevant range of concentrations.

The changes in the synaptic parameters derived from the biexponential fits for each of the enantiomers (fig. 6) qualitatively mirrored those seen with the racemate (fig. 4). For each enantiomer, the increase in total charge transfer and the extent of the stereoselectivity could be ascribed entirely to the slow component of the IPSC, with no significant effect on the fast component (fig. 6). Table 4 gives the percentage changes in the various synaptic parameters at a concentration of 25 μM for each of the two enantiomers.

Discussion

Lack of Effect of Racemic Thiopental on Excitatory Synaptic Currents

The absence of any effect of thiopental on excitatory glutamatergic synapses, even at concentrations significantly greater than those corresponding to surgical anesthesia, came as a surprise to us (although we later became aware of a study[20] on hippocampal slices showing a similar result). In fact, one of our reasons for using the optical isomers of thiopental was to test whether effects on glutamatergic or GABAergic synapses were stereoselective and, if so, to see which best matched the stereoselectivity observed in animals. We were able to carry out our plan only for the inhibitory but not for the excitatory synapses, because thiopental only affected the former. Indeed, even at a free (*i.e.*, unbound) aqueous concentration of 50 μM thiopental, anesthetic effects on glutamatergic synapses were insignificant (figs. 1 and 2 and table 2), yet at this free aqueous concentration the rat electroencephalogram is isoelectric[22,27] (80 μg/ml in plasma). For comparison, 10 μM thiopental is the concentration at which a rat[22] will lose its righting reflex and the concentration at which humans[28] fail to respond to a verbal command, whereas at approximately 25 μM thiopental, humans[28] or rats[22] will fail to respond to a painful stimulus (trapezius muscle squeeze or tail clamp).

Table 3. Effects of 25 μM Racemic Thiopental on Inhibitory Synaptic Currents

Parameter	% change (mean ± SEM)	↑, ↓ or NS*	n†
I_{peak}	−10.6 ± 1.9	↓	11
Decay half-time	367 ± 39	↑	11
Total charge transfer	231 ± 23	↑	11
I_{fast}	−46 ± 9	↓	10
I_{slow}	97 ± 35	↑	10
τ_{fast}	176 ± 62	↑	10
τ_{slow}	159 ± 31	↑	10
Q_{fast}	108 ± 80	NS	10
Q_{slow}	336 ± 46	↑	10

* NS = not significant at the 95% confidence level (Student *t* test); † n = number of cells.

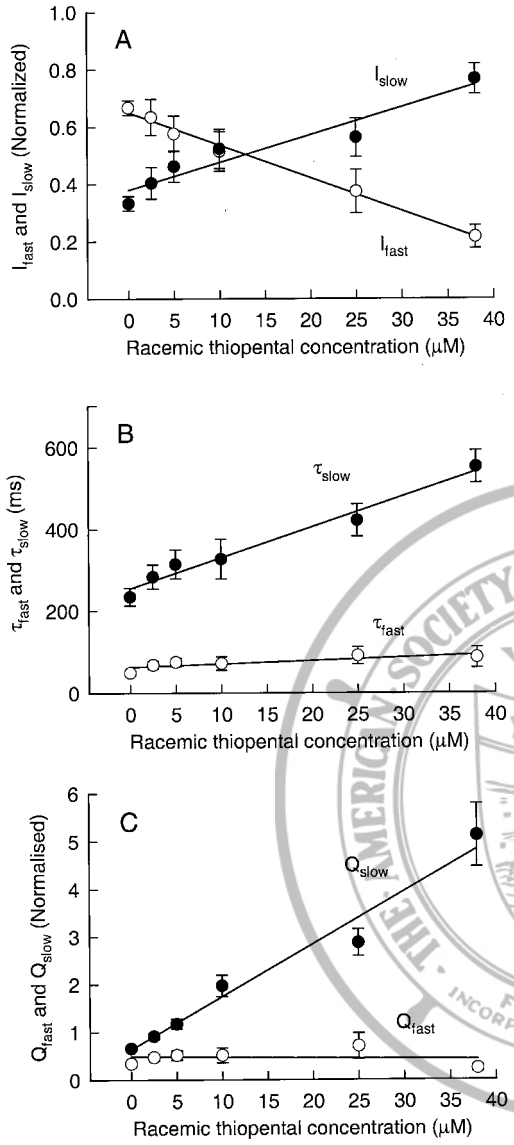

Fig. 4. The effects of thiopental on the fast and slow components of the inhibitory postsynaptic current derived from biexponential fits. (*A*) Changes in the amplitudes of the fast (open circles) and slow (filled circles) components, normalized such that the sum of the fast and slow components is equal to unity in the absence of thiopental. (*B*) Changes in the fast (open circles) and slow (filled circles) time constants. (*C*) Changes in the charge transfer for the fast (open circles) and slow (filled circles) components, normalized such that the total charge transfer for the control equals unity. The points represent mean values (for an average of 14 cells). In all cases the lines are drawn by eye and have no theoretical significance.

Therefore, our data do not support the idea that inhibition of glutamatergic synapses *per se* contributes to thiopental anesthesia. Moreover, the complete absence of any effect on this synaptic system is a powerful observation in that it implies that several putative molecular targets are unlikely to be important. Among these targets are, specifically, presynaptic calcium channels[29] (P/Q-type in these synapses[30,31]), the exocytotic machinery underlying neurotransmitter release, glutamate reuptake processes, and postsynaptic AMPA and NMDA receptors.

Significant inhibitions of AMPA and NMDA receptors have, however, been reported for thiopental[19,32] and other barbiturates.[15–18,32] In some cases, these positive reports can be explained by the use of concentrations that were not clinically relevant.[18,19] In other cases,[15–17,32] it is pos-

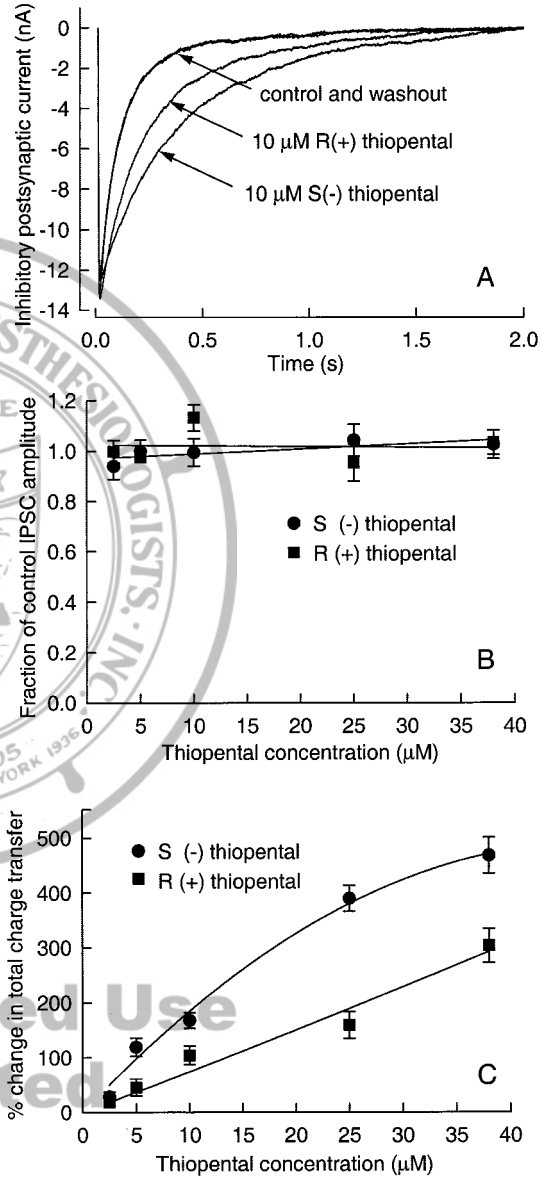

Fig. 5. Stereoselective effects of the thiopental enantiomers on inhibitory γ-aminobutyric acid–mediated (GABAergic) currents. (*A*) Representative traces showing the effects of 10 μM S(−) and R(+) thiopental on GABAergic synaptic currents. The S(−) enantiomer (circles) is significantly more effective than the R(+) enantiomer (squares) at prolonging the postsynaptic current decay (in this example, the decay half-times differ by approximately 2.4-fold). (*B*) The amplitude of the inhibitory postsynaptic current (IPSC) was not significantly affected by either enantiomer. (*C*) The increase in total charge transfer (the total area under the IPSC) is plotted as a percentage change from the control value for each of the enantiomers. The points represent mean values (for an average of 10 cells). In all cases the lines are drawn by eye and have no theoretical significance.

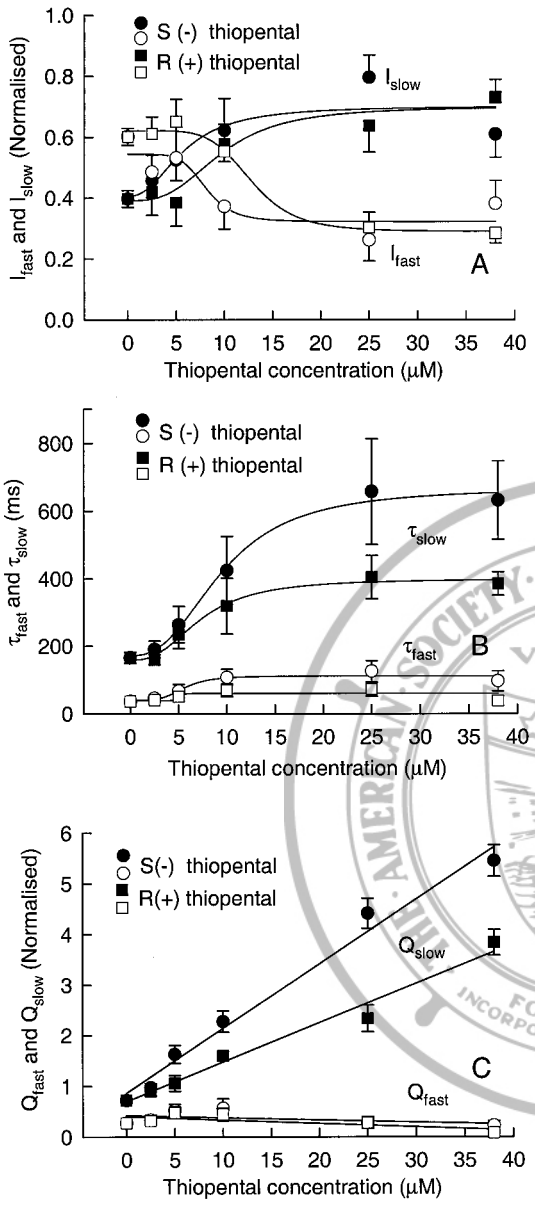

Fig. 6. The effects of the thiopental enantiomers on the fast and slow components of the inhibitory postsynaptic currents (IPSCs) derived from biexponential fits. (*A*) Changes in the amplitudes of the fast (open circles) and slow (filled circles) components, normalized such that the sum of the fast and slow components is equal to unity in the absence of anesthetic. (*B*) Changes in the fast (open circles) and slow (filled circles) time constants. (*C*) Changes in the charge transfer for the fast (open circles) and slow (filled circles) components, normalized such that the total charge transfer for the control equals unity. The points represent mean values (for an average of 13 cells). In all cases the lines are drawn by eye and have no theoretical significance.

sible that the experimental use of unphysiologic agonists may have given a misleading picture of receptor sensitivity. From our results, it seems clear that at functional synapses, any inhibition of postsynaptic glutamate receptors must be negligibly small at clinically relevant concentrations of thiopental. The suggestion that open-channel and hence "use-dependent" inhibition might be important[13,15] in enhancing glutamate receptor sensitivity to barbiturates is not supported by our data with thiopental. This can be seen by the fact that the time constants for EPSC decay, a sensitive measure of open-channel block, are unaffected (table 2), even at the highest concentration of thiopental used (50 μM), although a very slowly developing open-channel block cannot be discounted.

Finally, regarding the importance of glutamatergic transmission in barbiturate anesthesia, we note that work with convulsant and depressant barbiturate stereoisomers,[32] as well as a study[33] using a genetically modified mouse (lacking a particular glutamate receptor subunit) have provided additional arguments against a role for glutamatergic synapses in barbiturate anesthesia.

Effects of Racemic Thiopental on Inhibitory Currents

The effects of thiopental on inhibitory GABAergic synapses were in marked contrast to the lack of effect we had observed at excitatory synapses. Even at subanesthetic concentrations (≤10 μM), thiopental significantly prolonged the postsynaptic currents (fig. 3). At a free aqueous concentration (10 μM) that was equivalent to that which would cause loss of consciousness[5] or righting reflex,[22] the total charge transfer was increased by approximately 140%, and this increased to approximately 230% at a concentration (25 μM) that would prevent a response to a painful stimulus. This latter (approximately threefold) increase in charge transfer is very similar to that reported in hippocampal slices[20] and to that produced[34] by pentobarbital at 50 μM, a concentration which we calculate to be the equivalent value for this barbiturate to prevent a response to a painful stimulus.

This large increase in total charge transfer at a surgically relevant concentration is significantly greater than that produced by isoflurane (~70% at 1 minimum alveolar concentration) in an identical preparation.[26] From this comparison one can conclude that isoflurane is likely to be recruiting additional targets to exert its anesthetic effects. Indeed, in the same study[26] we observed a small but significant inhibition of glutamatergic synaptic transmission that probably contributes to isoflurane anesthesia, presumably together with other targets such as anesthetic-activated potassium channels.[35]

The lack of any effect of thiopental on the peak amplitude of the IPSC is consistent with the data (fig. 1 and table 2) on the EPSC and the notion that there are no significant presynaptic effects. In the case of GABAergic synapses, however, this conclusion is less secure because an inhibition of the peak amplitude might have been counteracted by a potentiation of postsynaptic receptors. In any event, the large prolongation in the IPSC decay is only reasonably interpreted in terms of a postsynaptic effect and is consistent with a large body of data in the literature[1–4] on the potentiating actions of

Table 4. Effects of 25 μM Thiopental Enantiomers on Inhibitory Synaptic Currents

	S(−) thiopental			R(+) thiopental		
Parameter	% change (mean ± SEM)	↑, ↓ or NS*	n†	% change (mean ± SEM)	↑, ↓ or NS*	n†
I_{peak}	4.4 ± 6.3	NS	7	−4.6 ± 7.0	NS	10
Decay half-time	396 ± 71	↑	7	196 ± 20	↑	10
Total charge transfer	390 ± 24	↑	7	159 ± 25	↑	10
I_{fast}	−47 ± 13	↓	7	−44 ± 9	↓	10
I_{slow}	113 ± 35	↑	7	53 ± 22	NS	10
τ_{fast}	143 ± 48	↑	7	53 ± 26	NS	10
τ_{slow}	226 ± 31	↑	7	128 ± 23	↑	10
Q_{fast}	1.4 ± 30.2	NS	7	1.4 ± 42.1	NS	10
Q_{slow}	511 ± 45	↑	7	223 ± 38	↑	10

* NS = not significant at the 95% confidence level (Student *t* test); † n = number of cells.

barbiturates on $GABA_A$ receptors (albeit rarely using thiopental).

One potentially interesting feature of the actions of thiopental on GABAergic synapses is the fact that the increase in total charge transfer can be entirely attributed to an increase in the slow rather than the fast component. We obtained the same result using isoflurane,[26] and this had also been reported previously for isoflurane by other investigators.[36,37] Although it is possible that the two components reflect little more than the complexities of channel kinetics,[38] the interesting possibility remains that this slow component represents a particular subtype of GABA receptor with an enhanced sensitivity to anesthetics.[39] However, the heterogeneity of receptor subtypes expressed at hippocampal autaptic synapses remains to be determined.

One caveat regarding the possible depression of glutamatergic synapses in the brain is that glutamatergic synapses might be functionally inhibited during thiopental anesthesia, but as a consequence of effects at presynaptic receptors (either by activation of $GABA_A$ receptors or inhibition of nicotinic acetylcholine receptors).[40,41] Indeed, a recent study showed that depolarization-evoked glutamate release from a cerebrocortical slice could be inhibited by thiopental, but that this inhibition could be attributed to the activation of $GABA_A$ receptors.[42]

Effects of Thiopental Enantiomers on Inhibitory Currents

Available evidence on the relative anesthetic potencies of the thiopental enantiomers in mice and rats show that the S(−) isomer is approximately twice as potent as the R(+) isomer.[9,43–45] We have argued[2] that the extent of stereoselectivity for a given anesthetic can be used as a powerful guide to which molecular targets contribute to a particular anesthetic end point and which do not. The data in figures 5 and 6 and table 4 show that the extent of the prolongation of the GABAergic postsynaptic current by thiopental is stereoselective, with the S(−) enantiomer also being approximately twice as effective as the R(+) enantiomer. This strongly supports the case for the $GABA_A$ receptor being a major target for thiopental. A similar conclusion emerged from two previous studies that examined the effects of thiopental enantiomers acting on defined $GABA_A$ receptor subunits expressed in either *Xenopus* oocytes[46] or PA3 cells.[8] In both cases, the stereoselectivity observed was roughly twofold, with the S(−) being again the more potent isomer. In contrast, we recently investigated[41] the effects of thiopental and its enantiomers on nicotinic acetylcholine receptors and found very different results. There we showed that, although these nicotinic receptors were very sensitive to inhibition, there was either no stereoselectivity or it was the opposite to that found for general anesthesia. From these data we concluded that nicotinic acetylcholine receptors are unlikely to play a major role in producing thiopental anesthesia.

Significance for Anesthetic Mechanisms

The significance of our results for understanding the mechanisms underlying thiopental general anesthesia are fairly clear. Given the extent of potentiation of GABAergic IPSCs at pharmacologically relevant concentrations, and given the close correspondence between the stereoselectivities of the *in vivo* potencies and the stereoselectivity in the effects on GABAergic synapses, we believe it would be accurate to say that the $GABA_A$ receptor is, very probably, the most important molecular target for thiopental. This is reinforced by our observations of no significant effects on glutamatergic synaptic transmission, which itself, as discussed above, rules out a number of other possible molecular sites of action. Although it always, of course, remains possible that new targets will be discovered, the most parsimonious working hypothesis is that thiopental causes general anesthesia by potentiating the actions of GABA at $GABA_A$ receptors.

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