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Stereoselective interaction of thiopentone enantiomers with the $GABA_A$ receptor

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1 As pharmacokinetic differences between the thiopentone enantiomers seem insufficient to explain the ~2 fold greater potency for CNS effects of (−)-S- over (+)-R-thiopentone, this study was performed to determine any enantioselectivity of thiopentone at the $GABA_A$ receptor, the primary receptor for barbiturate hypnotic effects.

2 Two electrode voltage clamp recording was performed on *Xenopus laevis* oocytes expressing human $GABA_A$ receptor subtype $\alpha_1\beta_2\gamma_2$ to determine relative differences in potentiation of the GABA response by *rac*-, (+)-R- and (−)-S-thiopentone, and *rac*-pentobarbitone. Changes in the cellular environment pH and in GABA concentrations were also evaluated.

3 With 3 μM GABA, the EC_{50} values were (−)-S-thiopentone (mean 26.0±s.e.mean 3.2 μM, $n=9$ cells) >*rac*-thiopentone (35.9±4.2 μM, $n=6$, $P=0.1$) >(+)-R-thiopentone (52.5±5.0 μM, $n=8$, $P<0.02$) >*rac*-pentobarbitone (97.0±11.2 μM, $n=11$, $P<0.01$). Adjustment of environment pH to 7.0 or 8.0 did not alter the EC_{50} values for (+)-R- or (−)-S-thiopentone.

4 Uninjected oocytes responded to >100 μM (−)-S- and R-thiopentone. This direct response was abolished by intracellular oocyte injection of 1,2-bis(2-aminophenoxy)ethane-N,N,N1,N1-tetraacetic acid (BAPTA), a Ca^{2+} chelating agent. With BAPTA, the EC_{50} values were (−)-S-thiopentone (20.6±3.2 μM, $n=8$) <(+)-R-thiopentone (36.2±3.2 μM, $n=9$, $P<0.005$).

5 (−)-S-thiopentone was found to be ~2 fold more potent than (+)-R-thiopentone in the potentiation of GABA at $GABA_A$ receptors expressed on *Xenopus* oocytes. This is consistent with the differences in potency for CNS depressant effects found *in vivo*.

Keywords: Thiopentone; enantiomers; $GABA_A$ receptor; *Xenopus* oocytes

Introduction

The main inhibitory neurotransmitter, γ-aminobutyric acid (GABA), activates the three major classes of GABA receptors: $GABA_A$, $GABA_B$ and $GABA_C$ (Johnston, 1996). The $GABA_A$ receptor is a ligand-gated chloride channel assembled from 14 different glycoprotein subunits (α_{1-6}, β_{1-3}, γ_{1-3}, δ and ε), in which 2α, 2β and one of either γ, δ or ε subunits combine to form a functional receptor complex (Tanelian *et al*., 1993). The majority of receptor subtypes found in most regions of the rat brain possess an α_1 subunit (Mohler *et al*., 1992). The $\alpha_1\beta_2\gamma_2$ receptor complex is also considered a dominant human receptor subunit combination *in vivo* (Lambert *et al*., 1997). Barbiturates primarily exert their sedative and anaesthetic effects by potentiation of the GABA response at $GABA_A$ receptors (Huang & Barker 1980; Leeb-Lundberg & Olsen 1982; Akaike *et al*., 1985; 1987; Peters *et al*., 1988; Thompson *et al*., 1996; ffrench-Mullen *et al*., 1993). Electrophysiological studies have shown that this potentiation is due to a prolongation in duration of the chloride ion channel open-time (Mathers & Barker, 1980). Barbiturates are also capable of direct activation of $GABA_A$ receptors and, at high concentrations, may block the chloride channel (Peters *et al*., 1988; Thompson *et al*., 1996).

Thiopentone differs from pentobarbitone by the replacement of oxygen with sulphur at the C2 position of the barbiturate ring. This substitution confers upon thiopentone different pharmacological properties, particularly a more rapid onset of action and a reduction in the terminal elimination half-life from 30–50 h to 10–15 h (Chan *et al*., 1985), that convey clinical advantages. Recent studies also suggest that *rac*-thiopentone possesses neuroprotective advantages over pentobarbitone due to *rac*-thiopentone's ability to inhibit nitric oxide induced cytotoxicity, and over propofol by means of *rac*-thiopentone's attenuation of NMDA- and AMPA-mediated glutamate excitotoxicity (Shibuta *et al*., 1998; Zhu *et al*., 1997). Thiopentone has also been shown to impair astrocyte glutamate uptake at concentrations relevant to its clinical use (Swanson & Seid, 1998). Nevertheless, the utility of prolonged high-dose *rac*-thiopentone therapy for neurological and neurosurgical emergencies as well as prolonged anaesthesia is limited by its association with cardiac depression and by a prolonged duration of effect related to non-linear pharmacokinetics (Stanski *et al*., 1980; Turcant *et al*., 1985; Le Corre *et al*., 1993; Russo *et al*., 1996).

Thiopentone is a racemate used as an equimolar mixture of (+)-R- and (−)-S-enantiomers; thus it should strictly be designated RS-, (±) or *rac*-thiopentone to designate its racemic nature. Pentobarbitone has the same configuration and signs of the optical rotations as thiopentone but was only used as the racemate. As the signs of the optical rotations are not of significance to this study, they have been deleted for simplicity. The potential for differences in the pharmacokinetics and pharmacodynamics of enantiomers of racemic drugs, including a number of barbiturates, is well known (Mather & Rutten, 1991). Despite its long history of clinical use, there has only been a limited study of the pharmacological differences between the thiopentone enantiomers. It has been reported that S-thiopentone is a more potent hypnotic than R-thiopentone in mice (Christensen & Lee 1973; Haley & Gidley

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1976), humans (Mark *et al*., 1977) and rats (Mather *et al*., 1999a). Although recent studies have identified various pharmacokinetic differences between R- and S-thiopentone (Mather *et al*., 1996, 1999c,d; Nguyen *et al*., 1996; Cordato *et al*., 1997, 1999), the differences would seem too small to explain the pharmacodynamic differences.

In this study, we investigated the relative differences in potentiation of the GABA response at GABA$_A$ receptor subtype $\alpha_1\beta_2\gamma_2$, expressed in *Xenopus laevis* oocytes, by *rac*-thiopentone, its R- and S- enantiomers and *rac*-pentobarbitone. We also evaluated the effects of changes in the pH of the cellular environment, as this may influence drug receptor binding along with alterations to the degree of drug ionization, and compared the EC_{50} values of R- and S-thiopentone and *rac*-pentobarbitone in the presence of lower (3 μM) and higher (15 μM) GABA concentrations.

Methods

Human GABA$_A$ receptor cDNAs

Human α_1, β_2 and γ_2 cDNA encapsulated in the pcDM8 vector (Stratogene, La Jolla, CA, U.S.A.) was obtained from Dr Paul Whiting (Department of Biochemistry and Molecular Biology, Neuroscience Research Centre, Merck, Sharp and Dohme Research Laboratories, Harlow, Essex, U.K.). The plasmids were linearized with NOT1 and mRNA were transcribed using T7 RNA Polymerase and capped with 5,7-methyl guanosine using the mMESSAGE mMACHINE kit from Ambion Inc. (Austin, TX, U.S.A.).

Drug preparations

Drugs used were: R- and S-thiopentone (Huang *et al*., 1996), *rac*-thiopentone sodium, *rac*-pentobarbitone sodium, γ-aminobutyric acid and 1,2-bis(2-aminophenoxy)ethane-N,N,N^1,N^1=tetraacetic acid [BAPTA] (Sigma Chemical Co, St Louis, MO, U.S.A.). R- and S-thiopentone (0.1 mmoles) were dissolved in 0.5 ml 4 M NaOH and 9.5 ml ND96 recording solution (mM) (NaCl 96, KCl 2, $MgCl_2.6H_2O$ 1, $CaCl_2$ 1.8, HEPES 5, pH 7.5) to give 10 mM solutions. Further dilutions with the same recording solution were performed to make the final concentrations used in the electrophysiological studies. *rac*-Thiopentone was prepared from a 50 : 50 mixture of the R- and S-thiopentone solutions and the electrophysiological findings were comparable to those obtained from *rac*-thiopentone sodium (Sigma). The pH of higher concentrations (>50 μM) of R- and S-thiopentone, *rac*-thiopentone and pentobarbitone was adjusted to pH 7.5 using 0.5–10 μl 2 M HCl, where appropriate.

Electrophysiological recording

Female *Xenopus laevis* were anaesthetized with 0.17% 3-aminobenzoic acid ethyl ester. A lobe of the ovaries was removed, rinsed with OR2 buffer (mM) (NaCl 82.5, KCl 2, $MgCl_2.6H_2O$ 1, HEPES 5, pH 7.5) and treated with collagenase A (2 mg ml^{-1} in OR-2, Boehringer Mannheim) for 2 h to separate oocytes from connective tissue and follicular cells. Released oocytes were then rinsed in ND96 storage solution (mM) (NaCl 96, KCl 2, $MgCl_2.6H_2O$ 1, $CaCl_2$ 1.8, HEPES 5, pH 7.5 supplemented with 2.5 mM pyruvate, 0.5 mM theophylline and 50 μM ml^{-1} gentamicin). Stage V to V1 oocytes were collected and stored in ND96 solution with constant mixing on an orbital shaker.

An amount of 50 ng 50 nl^{-1} of α_1, β_2 and γ_2 cRNA in the ratio of 1 : 1 : 1 was injected into the cytoplasm of defolliculated Stage V *Xenopus* oocytes. Oocytes were stored at 9°C in ND96 storage solution; 2–8 days after mRNA injection, receptor activity was measured by two electrode voltage clamp recording using a Geneclamp 500 amplifier (Axon Instruments Inc., Foster City, CA, U.S.A.), MacLab 2e recorder (AD Instruments, Sydney, NSW, Australia) and Chart version 3.5.2 software on a Macintosh Quadra 605 computer. Oocytes were placed in a cell bath and voltage clamped using two micropipettes containing 3 M KCl. The membrane potential was clamped at −60 mV and the oocyte continually superfused with ND96 recording solution. Before any recording, oocytes were screened for functional receptor formation by the addition of 3 μM and 100 μM GABA to the cell bath. Different concentrations of drugs being tested were added to the buffer solution for receptor activation measurements. The drugs were applied until a peak response was observed, followed by a 3 min washout period to avoid receptor desensitization.

pH adjustments

The pH of the buffer solutions containing different concentrations of R- and S-thiopentone was adjusted to pH 7.0 or 8.0 using small amounts (<0.1% buffer volume) of 2 M HCl or NaOH to determine any effect on the degree of GABA potentiation in the presence of 3 μM GABA.

Analysis of electrophysiological data

Current ('*I*') dose-response relationships were measured from the recorded peak amplitude of *I* obtained for each drug concentration. The parameters of the relationship between '*I*' and agonist concentration [A] were determined from the least squares fit of the Hill equation $'I' = 'I_{max}' [A]^{n_H}/(EC_{50}^{n_H} + [A]^{n_H})$ to the data (where 'I_{max}' is the maximal current, EC_{50} is the effective dose that activates 50% of the maximal current for n individual cells and n_H is the Hill coefficient). The EC_{50} values, expressed as the mean ± s.e.mean from a number of different cells (*n*), were calculated by fitting data from individual oocytes using Kaleidograph 2.1 software. The statistical significance of differences between mean EC_{50} values was determined by one-way analysis of variance (ANOVA).

Results

Expression of human $\alpha_1\beta_2\gamma_2$ mRNA in *Xenopus* oocytes generated GABA$_A$ receptors that showed a dose-dependent GABA-activated inward current when the cell was voltage clamped at −60 mV. The log dose-response curve gave an EC_{50} value of 32.1 μM (n=5 cells, s.e.mean 5.7) and a Hill coefficient of 1.3 (s.e.mean 0.1). The effects of R-thiopentone, S-thiopentone, *rac*-thiopentone and *rac*-pentobarbitone on the GABA$_A$ $\alpha_1\beta_2\gamma_2$ receptor subtype were studied in the presence of 3 and 15 μM GABA. The degree of potentiation was measured by subtracting the current ('*I*') produced by GABA, 3 or 15 μM respectively, in the absence of agonist drug from the absolute current ('*I*') value produced in the presence of agonist drug. The direct activation component of the agonist drug, however, was included in the potentiation response value. For R-thiopentone, S-thiopentone and *rac*-thiopentone, >90% of the maximal GABA potentiation response was achieved with 100 μM agonist drug concentrations. For *rac*-pentobarbitone, 300 μM concentrations were

required to achieve >90% of the maximal GABA potentiation response. In contrast, direct activation of the $GABA_A$ receptor by 100 μM S-thiopentone and R-thiopentone produced responses that were 13 and 5%, respectively, of the maximal GABA potentiation response.

Xenopus oocytes not injected with human $\alpha_1\beta_2\gamma_2$ mRNA were found also to respond to >100 μM concentrations of R- and S-thiopentone. This direct response was not seen with 1 mM *rac*-pentobarbitone. In uninjected oocytes, the respective median currents produced by 100 μM and 1 mM S-thiopentone were 15 nA (range 5–50 nA, $n=5$) and 150 nA (range 20–500 nA, $n=7$); the respective median currents produced by 100 μM and 1 mM R-thiopentone were 10 nA (range 2–20 nA, $n=5$) and 50 nA (range 20–200 nA, $n=5$). In *Xenopus* oocytes injected with human $\alpha_1\beta_2\gamma_2$ mRNA, the respective median currents produced by 100 μM and 1 mM R-thiopentone were 120 nA (range 5–260 nA, $n=11$) and 720 nA (range 140–1500 nA, $n=5$); the respective median currents produced by 100 μM and 1 mM R-thiopentone were 50 nA (range 2-115 nA, n = 10) and 250 nA (range 60–600 nA, $n=5$). Hence, a dose-related and enantioselective difference was apparent in the endogenous direct responses produced. Endogenous receptor responses, including muscarinic cholinergic and voltage-sensitive calcium-dependent chloride currents, have been previously described in *Xenopus* oocytes (Kusano *et al*., 1982; Miledi & Parker 1984). Atropine (1 mM) did not significantly alter the response of 1 mM R- or S-thiopentone on uninjected cells. Intracellular injection of 50 nl 50 mM BAPTA, a Ca^{2+} chelating agent that was injected into the *Xenopus* oocytes at least 30 min prior to electrophysiological recording, virtually completely abolished the direct response to 1 mM R- and S-thiopentone. Hence further electrophysiological experiments comparing the degree of potentiation of the 3 μM GABA response by R- and S-thiopentone were carried out in *Xenopus* oocytes injected intracellularly with 50 mM BAPTA.

Electrophysiological responses in the absence of intracellular BAPTA

The EC_{50} for potentiation of 3 μM GABA by R-thiopentone was significantly greater than the EC_{50} for *rac*-thiopentone and S-thiopentone ($P=0.02$ and $P=0.0004$, respectively). In contrast, the EC_{50} for *rac*-pentobarbitone was greater than that for R-thiopentone ($P=0.007$). The EC_{50} and Hill coefficient for *rac*-pentobarbitone in the presence of 15 μM GABA were both comparable to previously reported values (Thompson *et al*., 1996). The EC_{50} values for potentiation of 15 μM GABA by *rac*-pentobarbitone, R-thiopentone and S-thiopentone were significantly less than those for the potentiation of 3 μM GABA ($P=0.004$, $P=0.002$ and $P=0.04$, respectively) by each agonist. The ratios of 'I_{max}'/1 mM GABA responses, however, were similar for both 3 and 15 μM GABA. Figures 1 and 2 illustrate the typical current responses of R- and S-thiopentone. The concentration-response curves for R-thiopentone, S-thiopentone, *rac*-thiopentone and *rac*-pentobarbitone, respectively, are shown in Figure 3. The EC_{50} values, Hill coefficients and ratios of 'I_{max}'/1 mM GABA response are summarized in Table 1.

The results of an adjustment in the environment of the cell bath to pH 7.0 and to 8.0 are also shown in Table 1. Although the concentration-response curves showed a trend towards a shift to the left at the lower pH, the mean differences in EC_{50} values were not significantly different ($P=0.08$ and $P=0.27$ for R-thiopentone and S-thiopentone, respectively).

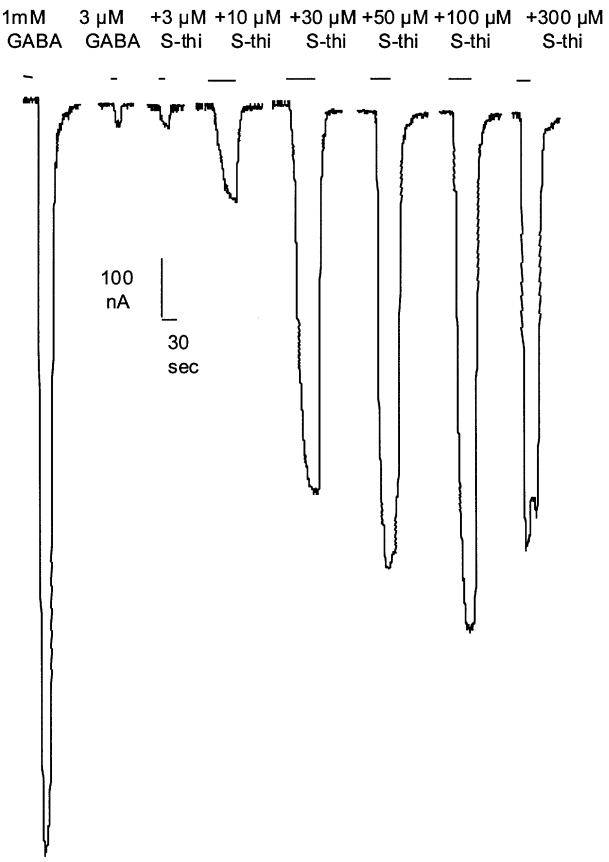

Figure 1 Illustrative recordings of current produced, in the absence and presence of S-thiopentone at increasing concentrations (as shown), by GABA at the $GABA_A$ receptor subtype $\alpha_1\beta_2\gamma_2$ expressed in *Xenopus* oocytes not injected with BAPTA.

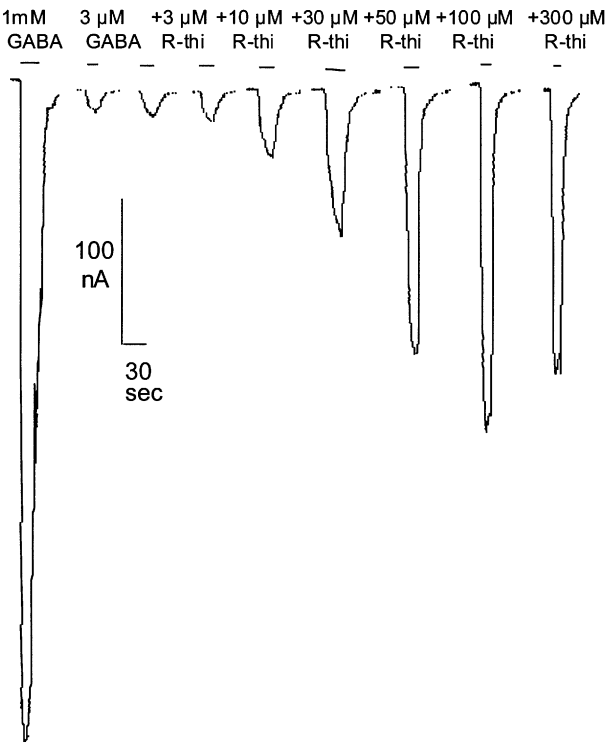

Figure 2 Illustrative recordings of current produced, in the absence and presence of R-thiopentone at increasing concentrations (as shown), by GABA at the $GABA_A$ receptor subtype $\alpha_1\beta_2\gamma_2$ expressed in *Xenopus* oocytes not injected with BAPTA.

Electrophysiological responses in the presence of intracellular BAPTA

The EC_{50} for potentiation of 3 μM GABA by S-thiopentone (20.6 ± 3.2 μM, $n = 7$ cells) was again significantly less than that for R-thiopentone (36.2 ± 3.2 μM, $n = 8$ cells, $P < 0.005$). The EC_{50} for potentiation of 3 μM GABA by R-thiopentone in the presence of BAPTA was significantly less than that for potentiation of 3 μM GABA in the absence of BAPTA ($P = 0.02$). The EC_{50} values, however, were not significantly different for S-thiopentone ($P = 0.20$).

Adjustment of the pH environment to 7.0, in the presence of BAPTA, did not significantly alter the EC_{50} values for R- and S-thiopentone when compared to those at pH 7.5. The EC_{50} values, Hill coefficient and ratio of 'I_{max}'/100 μM GABA responses at pH 7.0 and 7.5, in the presence of BAPTA, are summarized in Table 2.

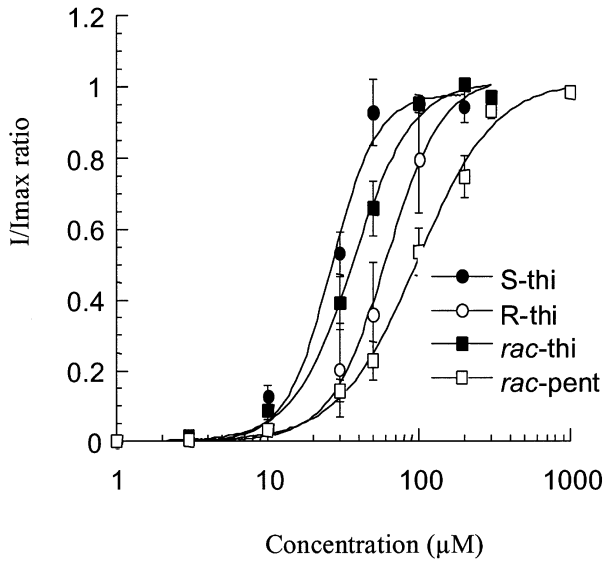

Figure 3 Dose-response relationships for rac-, R- and S-thiopentone and *rac*-pentobarbitone in the presence of 3 μM GABA at the GABA$_A$ receptor subtype $\alpha_1\beta_2\gamma_2$ expressed in *Xenopus* oocytes not injected with BAPTA.

Discussion

Intravenous *rac*-thiopentone remains the most widely used agent for the induction of anaesthesia after more than six decades of clinical use. During the last two decades, prolonged high dose *rac*-thiopentone aimed at achieving marked electroencephalographic (EEG) slowing or EEG burst-suppression activity has been introduced for treating acute neurological and neurosurgical emergencies, but is usually restricted to refractory cases due to haemodynamic side-effects and an unwanted and prolonged post-infusion duration of clinical unresponsiveness. We have been considering the question of whether enantiopure thiopentone might offer pharmacological advantages over the currently-used racemate. R-thiopentone is less potent than S-thiopentone in producing anaesthesia and lethality. It has been reported that the median anaesthetic and lethal doses for R-thiopentone in mice were approximately 2 fold greater than S-thiopentone (Christensen & Lee, 1973). Moreover, longer sleep duration times were

Table 1. Values of EC_{50}, Hill coefficients (n_H) and ratios of 'I_{max}'/1 mM GABA at the GABA$_A$ receptor subtype $\alpha_{1\beta_2\gamma_2}$ expressed in *Xenopus* oocytes not injected with BAPTA

*Agonist**	*GABA conc./pH†*	*EC_{50} (μM)#*	*Number of cells*	*n_H#*	*'I_{max}'/1 mM GABA#,##*
GABA	pH 7.5	32.1 ± 5.7	5	1.3 ± 0.1	1
S-thi	3 μM/pH 7.5	26.2 ± 3.3	9	2.7 ± 0.5	0.74 ± 0.1
R-thi	3 μM/pH 7.5	52.5 ± 5.0	8	2.3 ± 0.4	0.49 ± 0.1
R + S-thi	3 μM/pH 7.5	35.9 ± 4.2	6	3.2 ± 0.4	0.55 ± 0.1
R + S-pent	3 μM/pH 7.5	97.0 ± 11.2	11	2.4 ± 0.5	0.96 ± 0.2
S-thi	15 μM/pH 7.5	15.6 ± 3.0	4	1.9 ± 0.1	0.97 ± 0.2
R-thi	15 μM/pH 7.5	25.3 ± 4.2	6	1.6 ± 0.2	1.03 ± 0.2
R + S-pent	15 μM/pH 7.5	41.9 ± 6.0	6	1.7 ± 0.2	0.91 ± 0.2
S-thi	3 μM/pH 7.0	21.2 ± 3.3	8	1.9 ± 0.3	0.73 ± 0.1
R-thi	3 μM/pH 7.0	43.1 ± 4.1	9	2.5 ± 0.2	0.55 ± 0.1
S-thi	3 μM/pH 8.0	30.3 ± 3.1	4	2.2 ± 0.2	0.58 ± 0.1
R-thi	3 μM/pH 8.0	51.6 ± 7.4	4	2.5 ± 0.4	0.47 ± 0.1

*S- and R-thi refer to (−)-S- and R-(+)-thiopentone, respectively, and R + S-thi and R + S-pent refer to *rac*-thiopentone and *rac*-pentobarbitone, respectively. †GABA conc./pH refer to the GABA concentration that was present in combination with different concentrations of agonist drug and the pH of the drug solution/cell bath. #Data are the mean ± s.e.mean. ##'I_{max}' refers to the ratio of the maximal current produced by agonist drug in combination with the GABA concentration (defined in column 2) minus the current produced by that GABA concentration to the current produced by 1mM GABA.

Table 2 Values of EC_{50}, Hill coefficients (n_H) and ratios of 'I_{max}'/1 mM GABA at the GABA$_A$ receptor subtype $\alpha_1\beta_2\gamma_2$ expressed in *Xenopus* oocytes injected intracellularly with 50 mM BAPTA

*Agonist**	*GABA conc./pH†*	*EC_{50} (μM)#*	*Number of cells*	*n_H#*	*'I_{max}'/1 mM GABA#,##*
S-thi	3 μM/pH 7.5	20.6 ± 3.2	8	1.3 ± 0.3	0.76 ± 0.1
R-thi	3 μM/pH 7.5	36.2 ± 3.2	9	1.8 ± 0.1	0.39 ± 0.1
S-thi	3 μM/pH 7.0	22.0 ± 2.0	7	2.5 ± 0.4	0.60 ± 0.1
R-thi	3 μM/pH 7.0	40.6 ± 3.2	7	2.2 ± 0.4	0.44 ± 0.1

*S- and R-thi refer to (−)-S- and (+)-R-thiopentone, respectively. †GABA conc./pH refer to the GABA concentration that was present in combination with different concentrations of agonist drug and the pH of the drug solution/cell bath. BAPTA is 1,2-bis(2-aminophenoxy)ethane-N,N,N^1,N^1-tetraacetic acid. #Data are the mean ± s.e. mean. ##'I_{max}' refers to the ratio of the maximal current produced by agonist drug in combination with the GABA concentration (defined in column 2) minus the current produced by that GABA concentration to the current produced by 1 mM GABA.

found for S-thiopentone than R-thiopentone in three human subjects administered 300 mg of either enantiomer (Mark *et al*., 1977). It has also been found that R-thiopentone has a greater relative distribution into CNS tissues and lesser distribution into cardiac tissues in rats, and this probably contributes to its higher therapeutic index (Mather *et al*., 1999b). The present studies indicate that the lower potency of R-thiopentone may be related to its lesser activity at the $GABA_A$ receptor.

Barbiturates modulate the $GABA_A$ receptor to enhance GABA-mediated inhibition primarily by increasing the channel mean open duration. *In vitro* radioligand binding studies with [^{3}H]-dihydropicrotoxinin, [^{35}S]-t-butylbicyclophosphorothionate ([^{35}S]-TBPT, a convulsant that binds to the picrotoxinin-binding site of the GABA-receptor complex), and [^{3}H]-diazepam have revealed differences in efficacy among a number of barbiturate derivatives (Leeb-Lundberg & Olsen 1982; Johnston 1983; Lohse *et al*., 1987; Olsen *et al*., 1986; Richter & Holtman 1982). For example, it has been found that the relative potencies (IC_{50} values) for the inhibition of [^{35}S]-TBPT binding increase in the order *rac*-methohexitone, *rac*-thiopentone, *rac*-pentobarbitone, *rac*-secobarbitone, *rac*-hexobarbitone, amobarbitone and phenobarbitone (Lohse *et al*., 1987). Stereoselective differences in potency for individual barbiturates are also known. The relative potencies for *rac*-pentobarbitone and its enantiomers in potentiation of GABA responses in frog sensory neurons and cultured mammalian neurons are S-pentobarbitone > *rac*-pentobarbitone > R-pentobarbitone (Huang & Barker 1980; Akaike *et al*., 1987).

The Hill slopes for both R- and S-thiopentone exceeded unity; this is consistent with the binding to the $GABA_A$ receptor of more than one agonist molecule to produce a GABA-evoked response (Colquhoun, 1998). The finding is similar to that previously reported for pentobarbitone (Thompson *et al*., 1996), and most probably reflects cooperativity of binding of agonist molecules to the $GABA_A$ receptor. Despite significant differences in EC_{50} values for R- and S-thiopentone, no enantiomeric differences in Hill coefficients were found.

It has also been recently shown that differences in $GABA_A$ subunits influence the degree of drug efficacy obtained. The human $GABA_A$ $\alpha_1\beta_2\gamma_2$ subunit combination, as used in the present study, is thought to be a dominant combination *in vivo* (Lambert *et al*., 1997). The $GABA_A$ receptor complex, however, is a multigene family. Thompson *et al*. (1996) described the influence of various α and β subunits on the effects of *rac*-pentobarbitone. They found that $GABA_A$ receptors containing α_6 subunits, that are believed to be primarily located on cerebellar granule cells, had a higher affinity and efficacy for the direct activation from *rac*-pentobarbitone than did other α subunits. In addition, the direct action of *rac*-pentobarbitone was not influenced by differences in β subunits to the same extent as the types of α subunits.

We found the relative potencies (EC_{50} values) to be S-thiopentone < *rac*-thiopentone < R-thiopentone. The finding of a $\simeq$2 fold higher EC_{50} for R-thiopentone than S-thiopentone supports other studies describing a similar difference in hypnotic potency (*vide supra*). Our study also showed that *rac*-thiopentone is $\geqslant$2-fold more potent than *rac*-pentobarbitone in its potentiation of the GABA response, a finding that is consistent with previous receptor binding studies. It is of interest that the EC_{50} for the GABA response of *rac*-thiopentone (35 μM) in our study is comparable to the free fraction associated with a serum concentration of 200 μmol.l^{-1} with the degree of protein binding at $\sim$80% (Morgan *et al*., 1981). This serum concentration clinically is associated with marked EEG slowing as well as a loss of motor activity to profound noxious stimuli (Hung *et al*., 1992).

A dose-dependent response to different GABA concentrations in the presence of a fixed concentration of pentobarbitone has been previously reported (Parker *et al*., 1986; Levitan *et al*., 1988). Our findings similarly demonstrate a leftward shift of the agonist dose-response curves for R- and S-thiopentone, as well as of *rac*-pentobarbitone, in the presence of a higher GABA concentration. A thiopentone enantiomeric difference in potency, however, is still apparent under these conditions. The introduction of vigabatrin, a GABA transaminase inhibitor, and of tiagabine, a GABA transport reuptake inhibitor, has resulted in the clinical availability of anticonvulsants that primarily act by increasing brain GABA levels. Free cerebrospinal fluid GABA concentrations in humans, for example, have been shown to increase 2–4 fold after a 2 month treatment period of oral vigabatrin (Ben-Menachem 1989). Hence it is conceivable that R- and S-thiopentone could exert a greater hypnotic effect if administered in the clinical setting of refractory status epilepticus to patients who are concurrently taking vigabatrin or tiagabine.

The effect of acidosis on the disposition of thiopentone has been studied previously. Brodie *et al*. (1950) reported that a pH of 6.8, induced by hypercarbia, resulted in decreased plasma thiopentone concentrations in dogs, and this was postulated have been caused by increased transfer of nonionized thiopentone into tissues. Hence, by increasing the uptake of thiopentone into brain and/or cerebrospinal fluid, decreased pH could enhance the anaesthetic effect by a pharmacokinetic mechanism. However, it was previously unknown if a change in nonionized drug concentrations could also influence the pharmacodynamic effect at a receptor level. Our study failed to demonstrate a significant change in EC_{50} values for either R- or S-thiopentone following a change in the cellular environment from pH 7.5 to 8.0 or pH 7.5 to 7.0.

In conclusion, S-thiopentone was found to be $\sim$2 fold more potent than R-thiopentone at potentiating the effects of GABA on $GABA_A$ receptors expressed on *Xenopus* oocytes. This difference is comparable to differences in hypnotic potency found previously in a small number of laboratory and clinical studies, most of which were performed over 20 years ago. Recent studies, however, have also indicated that relative lethal potential of S-thiopentone is increased disproportionately to R-thiopentone (Mather *et al*., unpublished observations). Further studies may be of value in determining whether enantiopure thiopentone has a clinical pharmacological advantage over *rac*-thiopentone.

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