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Synaptic Mechanisms of Thiopental-induced Alterations in Synchronized Cortical Activity

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Background: **Anesthetic depth after barbiturate administration has been correlated with distinct electroencephalogram (EEG) patterns. The current study used a rat neocortical brain slice micro-EEG preparation to investigate synaptic mechanisms underlying thiopental-induced transitions in synchronized neuronal activity.**

Methods: **Concentration-dependent cellular actions of thiopental were investigated in brain slices using specific pharmacologic probes, whole cell patch clamps, and extracellular field recordings. θ-Like micro-EEG oscillations were elicited in neocortical slices by mimicking subcortical cholinergic and gamma-aminobutyric acid (GABA) afferent input with carbachol (100 μM), a cholinergic agonist, and bicuculline (10 μM) a $GABA_A$ antagonist.**

Results: **In the presence of 20 μM thiopental, micro-EEG slowing from θ (7.3 ± 0.9 Hz, mean ± SD, n = 19) to δ frequencies (2.5 ± 0.5 Hz, n = 11) was associated with a threefold prolongation of inhibitory currents. Burst suppression activity occurred at 50 μM thiopental, and appeared to result from direct activation of $GABA_A$-gated chloride currents, observed with voltage clamp recordings, and mimicked with a direct acting $GABA_A$ agonist, muscimol (1 μM). Isoelectric activity occurred at 100 μM thiopental, and likely resulted from reduced glutamatergic transmission, evidenced by depressed excitatory postsynaptic potentials. Glutamatergic excitation was required for burst suppression activity, because glutamate receptor antagonists blocked thiopental-induced bursts; forcing a transition to isoelectric activity.**

Conclusions: **Thiopental produced a continuum of EEG-like states in brain slices similar to those observed *in vivo*. The progression of thiopental-induced effects appear to have resulted from specific cellular actions that were recruited in a concentration-dependent manner. Progressive enhancement of synaptic inhibition followed by depression of excitatory transmission led to micro-EEG frequency slowing, burst suppression, and isoelectric activity. (Key words: Anesthetics, barbiturates: thiopental. Brain: γ-aminobutyric acid; hippocampus; neocortex. Monitoring, electroencephalogram: burst suppression; isoelectric electroencephalogram activity; oscillations. Monitoring, electroencephalographic frequency bands: alpha; delta; theta. Receptors: glutamate; muscarinic.)**

ALTHOUGH a variety of cellular actions have been associated with barbiturate anesthesia,[1–6] it remains unclear which actions are most relevant for achieving and maintaining progressively deeper levels of anesthesia. Previous studies have shown that thiopental-induced electroencephalographic (EEG) alterations can be correlated with behavioral measures of anesthetic depth.[7–11] During wakefulness, 3.5–7.5 Hz θ rhythm activity dominates rat EEG activity. Loss of tail pinch reflex occurs when EEG activity slows into the δ range (0.5–3.5 Hz). Loss of corneal reflex occurs during EEG burst suppression, and isoelectric activity is required for blocking reflex responses to intubation.[11] The ability to generate synchronous EEG-like activity using *in vitro* preparations[12–15] prompted the current study investigating thiopental actions on micro-EEG activity in neocortical rat brain slices in an attempt to link synaptic mechanisms of action with the continuum of EEG effects observed during thiopental anesthesia.

Materials and Methods

Two separate experimental protocols were used in the current study. EEG-like activity was pharmacologically evoked in neocortical regions, while patch clamp and evoked field excitatory postsynaptic potential (EPSP) recordings were performed in hippocampal cortex. Hippocampal rather than neocortical neurons were patched because of difficulties associated with eliciting synchronous monosynaptic evoked inhibitory postsynaptic currents (IPSCs) in neocortex, and because of difficulties associated with patching onto sparsely distributed neocortical somata. It was shown that thiopental produced similar effects on hippocam-

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pal and neocortical EEG activity, as shown in the preceding *in vivo* article.[10] Previous studies have shown that θ EEG activity in hippocampal slices[12–14,16] shares a similar frequency (3.5–7.5 Hz.), amplitude (50–250 μV), and pharmacologic profile with the neocortical ξ-like oscillations observed in the current study.

Slice Preparation

Experiments were performed on slices isolated from juvenile male Sprague-Dawley rats (weighing 80–120 g). Experimental protocols were approved by the Institutional Animal Care Committee at Stanford University and adhered to published guidelines of the National Institutes of Health, Society for Neuroscience, and American Physiological Society. Rats were anesthetized with diethyl ether and their hearts were stopped with a blow to the back of the thorax. Brains were removed to cold (1–2°C) oxygenated artificial cerebrospinal fluid (ACSF, see Materials). Brains were sectioned in the coronal plane into 450-μm thick slices using a vibratome (Vibraslice, Boston, MA). Slices containing both neocortical and hippocampal areas were then hemisected and placed on filter papers at the interface of a humidified carbogen (95% O_2/5% CO_2) gas phase and ACSF liquid phase. Slices were allowed at least 1 h to recover from the slicing procedure before submersion in ACSF in a recording chamber. The ACSF was saturated with carbogen gas and perfused at a rate of 2.5 ml/min, at room temperature (21–24°C). Rapid and accurate solution changes were made using a ValveBank8 computerized perfusion system (AutoMate Scientific, Oakland, CA). Thiopental concentrations were measured using high-performance liquid chromatography.[17]

Micro-electroencephalogram Generation, Recording, and Analysis

In vivo θ EEG activity has been shown to be associated with activation of both ascending cholinergic and GABAergic inputs.[18] Cholinergic inputs are thought to depolarize pyramidal neurons, whereas GABAergic inputs have been shown to selectively innervate inhibitory neocortical interneurons,[19,20] suggesting that activation of these GABAergic afferents results in neocortical disinhibition. These endogenous inputs were mimicked in neocortical slice micro-EEG experiments by using ACSF containing the cholinergic agonist carbachol (100 μM), and the $GABA_A$ antagonist bicuculline (10 μM). γ-Aminobutyric acid (GABA) was not used because in addition to blocking inhibitory interneuron activity, it would directly inhibit pyramidal neurons and interfere with cholinergic excitation of these cells.

Pharmacologically evoked EEG-like oscillations were recorded with low resistance (<2 MΩ) glass microelectrodes filled with ACSF and placed in layer 2 or 3 of the neocortex for most experiments (fig. 1). These micro-EEG signals were amplified ×10,000–50,000 (model 210A; Brown-Lee Precision, San Jose, CA), filtered 1–30 Hz band-pass, 60 Hz notch (Cyber Amp 380, Axon Instrument, Foster City, CA), digitized (256 or 2,048 Hz; DataWave Technologies, Longmont, CO) and stored on computer disk for further analysis. Micro-EEG spectral analysis was accomplished using fast Fourier transforms on 2.5-s long epochs of data using DataWave software.

Micro-electroencephalogram Pharmacology

Before drug application, each neocortical slice displayed a 20-min baseline consisting of trains of spontaneous θ-like micro-EEG activity in ACSF containing carbachol (100 μM) and bicuculline (10 μM). All pharmacologic agents were applied in ACSF containing carbachol and bicuculline, and drug application was continued until steady-state effects were achieved (*i.e.*, burst suppression or isoelectric activity). Drug washout began within 15 min of achieving a steady-state effect.

Single Cell Recording

Whole cell recording microelectrodes (4–8 MΩ) contained an internal solution comprising (in mM): K-gluconate 100; ethylene glycol-bis(β-amino-ethyl ether) N,N,N′,N′-tetra acetic acid 10; $MgCl_2$ 5; N-[Z-hydroxyethyl]piperazine-N′-[Z-ethane sulfonic acid] (HEPES) 40; adenosine triphosphate 0.3; and guanosine triphosphate 0.3, *p*H = 7.2 and osmolarity = 280–290 mOsm. Monosynaptic IPSCs were evoked in stratum radiatum with a bipolar-stimulating electrode (5 V, 250 μs, 0.033 Hz), in the presence of 6-cyano-7-nitroquinoxaline-2,3-dion and (±)-2-amino-5-phosphonovaleric acid (see Materials) to block excitatory glutamate-mediated transmission. Stimulating electrodes were placed for optimal $GABA_A$, slow IPSC activation.[21] Cells were voltage clamped at −60 mV (Axoclamp 2A, Axon Instrument), which is typical for resting membrane potentials in hippocampal pyramidal cells.[22] Signals were amplified (×500), filtered (DC—10 kHz band-pass; Axon Instrument), digitized (10 kHz; DataWave Technologies) and stored on computer disk for further analysis.

Hippocampal Field Recordings

Field EPSPs were evoked (3–8 V, 250 μs, 0.1 Hz) with a bipolar tungsten stimulating electrode placed in stratum

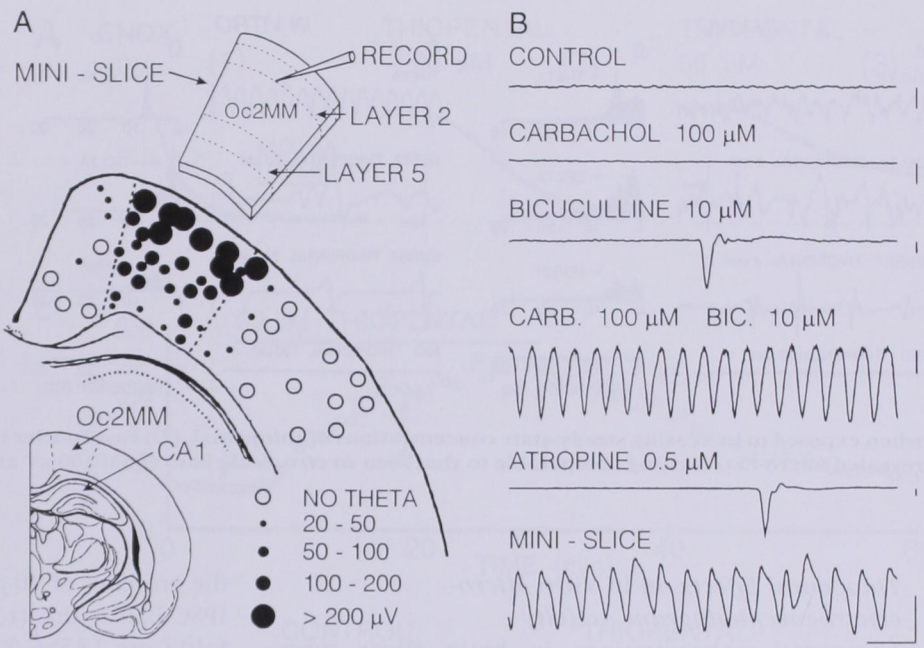

Fig. 1. Characterization of θ-like EEG generator in neocortex (area Oc2MM). (*A*) Micro-EEG recording positions in a hemisected coronal brain slice map the presence (●) or absence (○) of neocortical θ frequency oscillations. Larger symbols represent higher amplitude activity. θ-like activity was observed primarily in cortical area Oc2MM, and oscillation amplitudes were greatest in superficial cortical layers 1, 2, and 3. (*B*) Two second voltage traces show the absence of micro-EEG activity under control conditions and in the presence of a cholinergic agonist, carbachol (100 μM). Bicuculline (10 μM), a $GABA_A$ antagonist, elicited only large amplitude spike activity. Sinusoidal θ-like oscillations were generated by simultaneous application of both carbachol and bicuculline. θ activity was abolished by the muscarinic receptor antagonist, atropine (0.5 μM), leaving only bicuculline-mediated events. A dissected mini-slice containing only area Oc2MM displayed spontaneous θ frequency oscillations, demonstrating the presence of an intrinsic micro-EEG generator in this cortical region. Scale bars equal 50 μV and 200 ms, respectively.

radiatum of area CA1. EPSPs were recorded with low resistance (<2 MΩ) glass electrodes filled with ACSF also placed in stratum radiatum.[23] Signals were amplified (×5,000), filtered (DC to 10 kHz) and digitized.

Materials

Rats were obtained from Simonsen Laboratories (Gilroy, CA). Thiopental, carbamylcholine chloride (carbachol), and atropine sulfate were obtained from Sigma (St. Louis, MO). (−)-Bicuculline methiodide, muscimol HBr, (±)-2-amino-5-phosphonovaleric acid and 6-cyano-7-nitroquinoxaline-2,3-dione were supplied by Research Biochemicals International (Natick, MA). All solutions were made with spectrophotometric grade water (Omnisolve) supplied by EM Science (Gibbstown, NJ). The ACSF had the following ionic composition (in mM): Na^+ 151.25; K^+ 3.5; Ca^{++} 2.0; Mg^{++} 2.0; Cl^- 130.5; HCO_3^- 26; SO_4^- 2.0; $H_2PO_4^-$ 1.25; and glucose 10. Chemicals for the ACSF were reagent grade or better and obtained from J.T. Baker (Philadelphia, PA).

Results

Neocortical θ Frequency Generator

Spontaneous trains of neocortical θ-like micro-EEG activity, lasting up to 9 s and ranging in amplitude from 20 to 450 μV, appeared *in vitro* during bath application of carbachol and bicuculline (fig. 1). These θ trains occurred 0.5–2 times per minute and, like cholinergically driven type II θ *in vivo*,[18] were blocked by the muscarinic receptor antagonist atropine (0.5 μM). This θ-like micro-EEG activity was not observed throughout the entire neocortex, rather it was confined to a bilateral strip of medial occipital cortex that runs rostrocaudally and included four anatomically defined areas: 29d, 18b, 17, and 18a,[24] also known as the occipital association areas: Oc2MM, Oc2MM/Oc2ML, Oc1M/Oc1B, and Oc2L, respectively.[25] Previous *in vivo* studies also have demonstrated the occurrence of θ EEG activity within these anatomically defined cortical regions.[26,27] The neurons and synchronizing circuits generating these θ frequency oscillations must be intrinsic to neocortex because isolated mini-slices of Oc2MM cortex produced θ-like activity, and paired electrode differential recordings revealed a phase reversal in this cortical region, indicating the presence of a local neuronal generator. The narrow bandwidth and sinusoidal nature of *in vitro* neocortical micro-EEG activity (fig. 1) may be caused by removal of other ascending modulatory influences such as catecholamine or indolamine afferents.

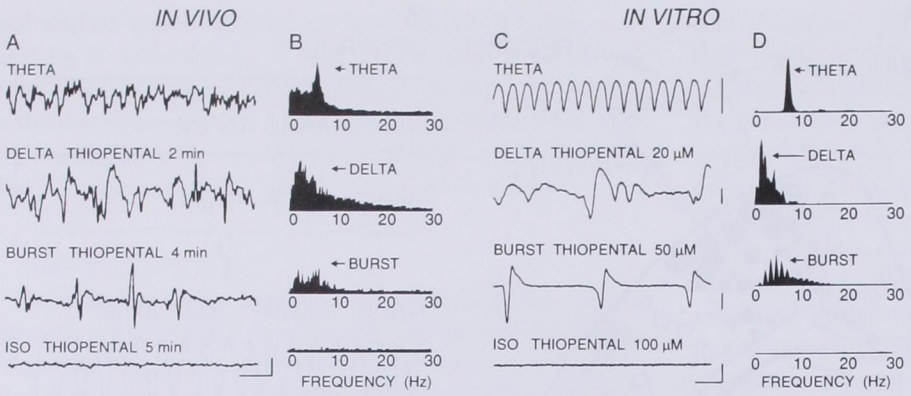

Fig. 2. Thiopental produced three distinct transitions in EEG spectra *in vivo* and *in vitro*. (*A*) *In vivo* recordings (2 s) from experiments described in MacIver *et al.*[10] show a thiopental-induced progression of EEG effects: $\theta > \delta >$ BURST (burst suppression) > ISO (isoelectric). These EEG recordings were obtained at various times during a thiopental infusion (10 mg/kg/min, for ~5 min). (*B*) Fast Fourier transforms of the waveforms displayed in (*A*) show a progressive slowing of EEG peak frequency with increasing concentrations of thiopental. (*C*) Recordings in neocortical brain slices displayed a similar progression of micro-EEG patterns when exposed to increasing steady-state concentrations of thiopental. (*D*) Fast Fourier transform analysis of *in vitro* recordings revealed micro-EEG slowing comparable to that seen *in vivo*. Scale bars equal 100 μV and 200 ms, respectively.

Thiopental Effects on In Vitro *Micro-electroencephalogram Activity*

Thiopental concentrations in brain slices were matched to calculated *in vivo* thiopental levels that produced progressively deeper stages of anesthesia.[9,10] In brain slices, fast Fourier transform analysis revealed that 20 μM thiopental produced a threefold decrease in micro-EEG frequency, from θ (7.3 ± 0.9 Hz; mean ± SD; n = 19) to δ (2.5 ± 0.5 Hz, n = 11). This decrease was comparable to a threefold slowing of EEG frequencies observed *in vivo* (fig. 2). Slice micro-EEG amplitudes increased ~375% during transitions from θ to δ frequency oscillations, also similar to results obtained *in vivo*.[10] Higher concentrations of thiopental (50 μM; n = 11) produced burst suppression micro-EEG patterns, characterized by large amplitude (200–500 μV) burst discharges separated by brief periods of isoelectric activity (figs. 2A and 2C). Burst activity in slices was either monophasic or biphasic with a sharp negativity followed by a low amplitude positive overshoot. *In vitro* bursts were typically separated by 0.5–3 s, whereas *in vivo* bursts were more varied (separated by 0.1–5 s). Increasing thiopental levels to 100 μM produced isoelectric activity, which reversed to θ-like activity after barbiturate washout (5 of 5 brain slices). Thus, a similar progression in EEG profiles was observed *in vivo* and *in vitro* over a clinically relevant concentration range.

Thiopental Prolongation of Inhibitory Currents Produced Micro-electroencephalogram Slowing

Thiopental effects on inhibitory currents were examined using whole cell patch clamp recordings. In the presence of 20 μM thiopental, monosynaptic evoked IPSC half width ($t_{1/2}$) increased approximately threefold from 135 ± 22 ms (n = 8) to 400 ± 40 ms (n = 5), whereas IPSC amplitudes remained relatively unchanged (figs. 3A and 3B). This threefold increase in IPSC $t_{1/2}$ was associated with the threefold slowing in micro-EEG frequencies observed during θ to δ transitions also produced by 20 μM thiopental (fig. 2).

To compare thiopental effects on IPSCs with effects on micro-EEG slowing, θ and δ rhythms were approximated as sine waves (fig. 3C). θ and δ sine wave periodicities of 137 and 397 ms were calculated from experimental mean θ (7.3 Hz) and δ (2.5 Hz) oscillation frequencies, respectively. Modeled EEG waves were superimposed on IPSCs recorded in the presence or absence of 20 μM thiopental (fig. 3C). Control and thiopental-prolonged IPSC amplitudes were compared at times corresponding to the periodicity of either θ or δ frequency oscillations, and revealed that both IPSCs had decayed ~38% from their peak values over these time periods (fig. 3C). The inhibitory current amplitude (~140 pA) associated with these time points may represent a critical degree of inhibition such that EEG generating neurons discharge and then remain inhibited until recurrent IPSCs decay below this critical level (*i.e.*, after 137 ms in control conditions *vs.* 397 ms in the presence of 20 μM thiopental). Thus, micro-EEG oscillation frequency appeared to depend on IPSC decay times. The thiopental-induced increase in variability of IPSC $t_{1/2}$ (fig. 3B) was also apparent in the increased standard deviation for δ micro-EEG activity (fig. 3C), as expected if IPSC prolongation resulted in the observed decrease in micro-EEG frequencies.

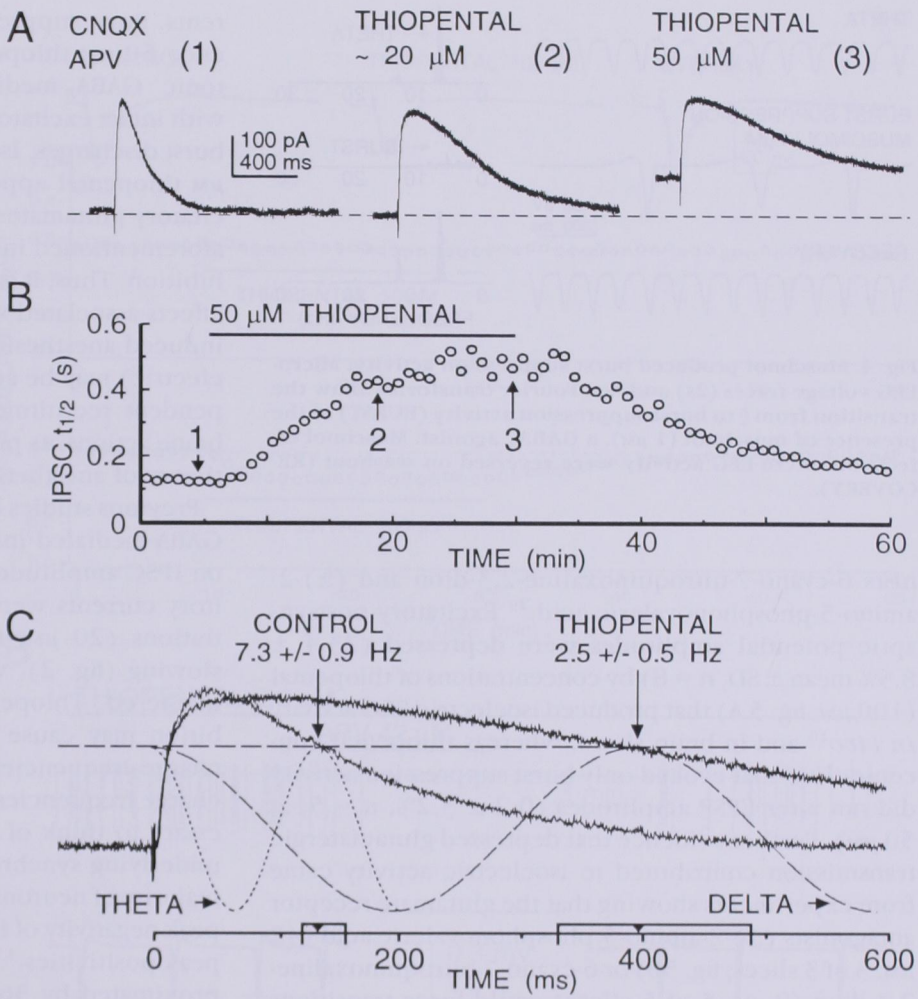

Fig. 3. Whole cell recordings demonstrated a thiopental-induced inhibitory postsynaptic current (IPSC) prolongation followed by direct activation of inhibitory currents. (*A*) Monosynaptic evoked IPSCs (1) were isolated using glutamate receptor blockers 6-cyano-7-nitroquinoxaline-2,3-dion (8.6 μM) and (±)-2-amino-5-phosphonovaleric acid (125 μM). During application of 50 μM thiopental, IPSC duration increased until a steady state was achieved. The trace displayed in (2) shows a presteady-state effect (~20 μM) of 50 μM thiopental on inhibitory postsynaptic current amplitude and duration. This recording was selected based on its similarity to effects observed in the presence of steady-state concentrations of 20 μM thiopental (see text). Under these conditions, IPSC $t_{1/2}$ increased approximately threefold. This prolongation was also observed at steady-state concentrations of 50 μM thiopental (3). In addition to prolonging IPSC $t_{1/2}$, 50 μM thiopental also produced a 98 pA positive shift in holding current necessary to maintain the voltage clamp at −60 mV. (*B*) Experimental time course of thiopental-induced IPSC $t_{1/2}$ increase (arrows indicate traces displayed in *A*), note the increased variability in $t_{1/2}$ produced by thiopental. (*C*) Comparison of IPSC time courses and micro-EEG periodicities. ξ and δ waveforms were plotted on control and thiopental prolonged IPSCs. The intercept point for each wave occurred at the same amplitude on the appropriate IPSC. The dashed line tangent to the peaks of both waveforms represents a critical amount of recurrent inhibition, above which EEG generating cells may be unable to discharge. The time bar shows the mean and SD for each micro-EEG waveform, and the increased variability in micro-EEG periodicity produced by thiopental during δ activity.

Hyperpolarization Underlies Thiopental-induced Burst Suppression Activity

Whole cell voltage clamp recordings revealed a tonic activation of inhibitory currents (~100 pA; fig. 3A) at thiopental concentrations (50 μM) that elicited sustained burst suppression micro-EEG activity. To test whether tonic $GABA_A$-mediated hyperpolarization contributed to burst suppression activity, micro-EEG effects of the $GABA_A$ agonist muscimol were studied. Muscimol (1 μM) produced a direct transition from θ to burst suppression activity without a slowing to δ frequencies (5 of 5 slices; fig. 4). The time course of this effect was rapid with bursts typically occurring 3–5 min after muscimol application, and recovery to θ-like activity occurring 2–4 min after drug removal. Muscimol-induced burst suppression activity was similar to burst suppression activity produced by 50 μM thiopental in that high amplitude (≥200 μV) monophasic or biphasic bursts occurred with an interburst interval of 0.5–3.0 s.

Depression of Excitatory Transmission Resulted in Isoelectric Activity

Thiopental effects on glutamate-mediated transmission were investigated using evoked field EPSPs in area CA1 of the rat hippocampus. These EPSPs are glutamate mediated,[28] and can be completely blocked by concurrent application of the glutamate receptor antago-

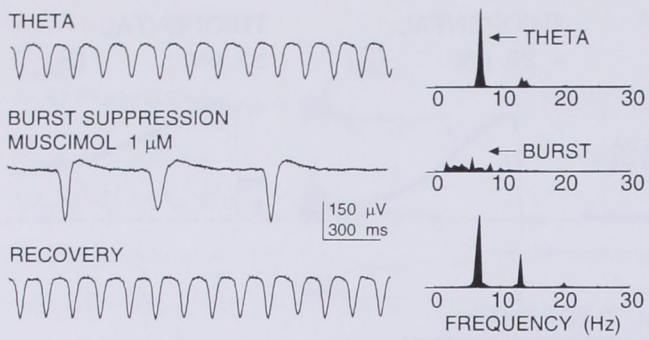

Fig. 4. Muscimol produced burst suppression activity. Micro-EEG voltage traces (2s) and fast Fourier transforms show the transition from ξ to burst suppression activity (BURST) in the presence of muscimol (1 μM), a $GABA_A$ agonist. Muscimol effects on micro-EEG activity were reversed on washout (RECOVERY).

nists 6-cyano-7-nitroquinoxaline-2,3-dion and (±)-2-amino-5-phosphonovaleric acid.[29] Excitatory postsynaptic potential amplitudes were depressed (23.4 ± 8.5% mean ± SD, n = 8) by concentrations of thiopental (100 μM; fig. 5 A) that produced isoelectric EEG activity *in vivo*[10] and in brain slices, whereas thiopental concentrations that evoked only burst suppression activity did not alter EPSP amplitudes (0.2 ± 3.2%; n = 5; at 50 μM). Further evidence that depressed glutamatergic transmission contributed to isoelectric activity came from experiments showing that the glutamate receptor antagonists (±)-2-amino-5-phosphonovaleric acid (42 μM, 3 of 3 slices; fig. 5B) or 6-cyano-7-nitroquinoxaline-2,3-dion (8 μM, 5 of 5 slices) could force transitions from burst suppression to isoelectric activity in the presence of 50 μM thiopental. This concentration of thiopental (50 μM) by itself did not produce isoelectric activity (0 of 10 slices).

Discussion

Anesthesia has been proposed to result from decreased excitatory transmission,[30–32] increased inhibitory transmission,[1,33–35] and/or inhibition of action potential generation *via* membrane hyperpolarization.[36–38] The current study demonstrated that each of these mechanisms could contribute to thiopental-induced alterations in synchronized neuronal activity. The threefold slowing in neocortical micro-EEG peak frequency from θ (7.3 Hz) to δ (2.5 Hz) activity, observed in the presence of 20 μM thiopental, was associated with a threefold prolongation of inhibitory currents. Burst suppression activity occurred in the presence 50 μM thiopental, and appeared to result from tonic $GABA_A$-mediated neuronal hyperpolarization, with intact excitatory transmission required to generate burst discharges. Isoelectric activity observed with 100 μM thiopental appeared to result from depressed excitatory glutamatergic transmission, in addition to the aforementioned increases in both phasic and tonic inhibition. Thus, it is possible that the continuum of EEG effects associated with deepening states of thiopental-induced anesthesia (θ > δ > burst suppression > isoelectric) may be accounted for by a concentration-dependent recruitment of separate synaptic and membrane actions, as predicted by a multisite agent-specific theory of anesthetic action.[23,39–41]

Previous studies have shown that anesthetics prolong GABA-mediated inhibition while exerting little effect on IPSC amplitudes.[1,34,42] In the current study, inhibitory currents were prolonged by thiopental concentrations (20 μM, fig. 3) that produced δ frequency slowing (fig. 2), whereas IPSC amplitudes remained unaltered. Thiopental-induced prolongation of inhibition may cause slowing by limiting neuronal discharge frequencies. To understand how limiting discharge frequencies could cause EEG slowing, it is necessary to think of the neuronal population dynamics underlying synchronous oscillatory EEG activity. The majority of neurons discharge preferentially during the peak negativity of EEG oscillations and are silent during peak positivities.[43] Thus, one full EEG oscillation (approximated by 360° of a sine wave, fig. 3C), likely represents the synchronous discharge, quiescence, and secondary discharge of a neuronal population. Stated another way, the length of time between neuronal population discharges determines the periodicity of an EEG oscillation. What then determines neuronal interdischarge time intervals? One likely candidate is the time course of recurrent GABA-mediated inhibition. In support of this, depression of GABA-mediated inhibition has been shown to elicit high-frequency discharge activity.[44] Conversely, high-frequency discharge activity associated with epilepsy can be suppressed by some barbiturates and other anesthetics[45] known to enhance GABAergic transmission. By prolonging the time course of inhibition, 20 μM thiopental may limit neuronal discharge frequencies, allowing lower frequency δ activity while filtering out higher frequency θ activity.

Previous studies have shown that burst suppression activity occurs during surgical anesthesia with thiopental.[7,9,11,46–48] In the current study, thiopental-in-

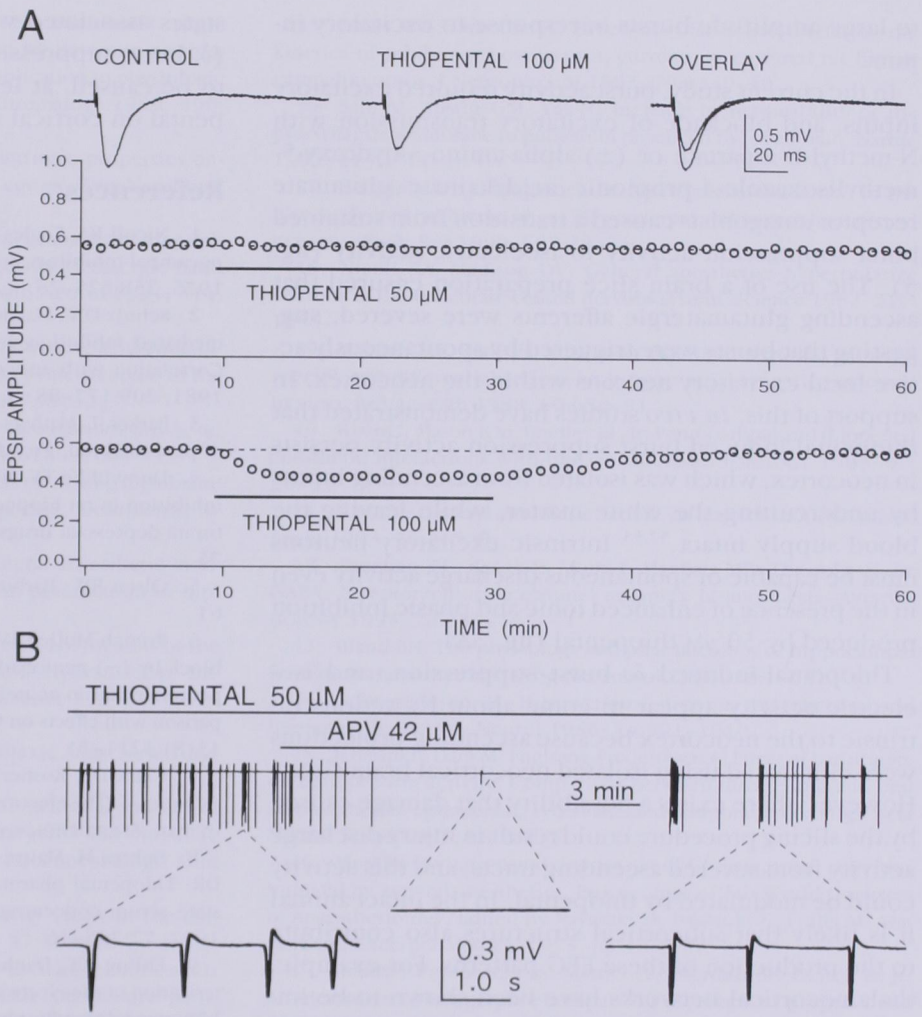

Fig. 5. Depression of glutamate-mediated transmission underlies the transition from burst suppression to isoelectric activity. (*A*) Data traces (*top*) show evoked field excitatory postsynaptic potentials (EPSPs) in area CA1 of the hippocampus in control conditions and in the presence of 100 μM thiopental. A clear depression in EPSP amplitude was observed when traces were overlaid. Plots display the time course of thiopental (50 and 100 μM) effects on EPSP amplitudes (*bottom*). Thiopental (50 μM) had no effect on EPSP amplitude, whereas 100 μM thiopental depressed EPSP amplitude by 23%. (*B*) Steady-state burst suppression activity was evoked and maintained in the presence of 50 μM thiopental. The NMDA receptor antagonist, D-(±)-2-amino-5-phosphonovaleric acid (42 μM), produced a transition to isoelectric activity which recovered to burst suppression on washout. Expanded time scale (*bottom*) shows individual burst events.

duced burst suppression-like activity was associated with increased tonic inhibition, evidenced by increased steady-state outward currents observed in the presence of 50 μM thiopental (fig. 3A). The ability of muscimol to produce burst suppression activity (fig. 4) demonstrated that enhanced tonic GABAergic hyperpolarization was sufficient to produce this micro-EEG state. In the presence of muscimol, θ-like oscillations progressed directly to burst suppression activity without passing through a δ state. δ activity should not and did not occur under these conditions because muscimol directly opens $GABA_A$-gated chloride channels without prolonging IPSC $t_{1/2}$[49]: supporting the idea that δ slowing resulted from a prolongation of inhibitory currents, whereas burst suppression activity required enhanced tonic inhibition (hyperpolarization).

These results are consistent with previous *in vivo* findings that demonstrated that neocortical neurons hyperpolarize (~10 mV) and increase their resting membrane conductance during anesthetic-induced burst suppression activity.[50] Increased tonic inhibition may contribute to burst suppression activity by hyperpolarizing EEG-generating neurons. Hyperpolarization would have three primary effects: (1) tonic cell discharge frequencies will decrease, reducing excitatory synaptic transmission between cortical neurons; (2) membrane potential-dependent inactivation will be removed from low threshold voltage activated calcium and sodium channels[51]; and (3) reduced cell discharge will leave fewer neurons refractory to firing. These conditions would favor a state in which EEG-generating neurons become both quiescent, leading to periods of suppressed EEG activity, and hyperexcitable, leading

to large amplitude bursts in response to excitatory inputs.

In the current study, burst activity required excitatory inputs, and blockade of excitatory transmission with N-methyl-D-aspartate or (±)-alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid/kainate glutamate receptor antagonists caused a transition from sustained burst suppression activity to isoelectric activity (fig. 5). The use of a brain slice preparation ensured that ascending glutamatergic afferents were severed, suggesting that bursts were triggered by spontaneously active local excitatory neurons within the neocortex. In support of this, *in vivo* studies have demonstrated that thiopental-induced burst suppression activity persists in neocortex, which was isolated from ascending inputs by undercutting the white matter, while leaving the blood supply intact.[52,53] Intrinsic excitatory neurons must be capable of spontaneous discharge activity even in the presence of enhanced tonic and phasic inhibition produced by 50 μM thiopental (fig. 3A).

Thiopental-induced δ, burst suppression, and isoelectric activity appear to come about by actions intrinsic to the neocortex because ascending projections were eliminated using isolated neocortical brain slices. However, there exists a possibility that damage caused by the slicing procedure could result in injury discharge activity from severed ascending tracts, and this activity could be modulated by thiopental. In the intact animal it is likely that subcortical structures also contribute to the production of these EEG patterns. For example, thalamocortical networks have been shown to be important in generating sustained δ activity during sleep.[54] One micro-EEG state not observed in neocortical brain slices was an early activation that preceded δ activity *in vivo,* and was characterized by increased power in θ, α, and β frequencies.[10] Consistent with a subcortical loci for EEG activation, previous studies have demonstrated the ability of thalamocortical,[54,55] septocortical, and pontocortical afferents[43,56] to activate cortical EEG signals *in vivo.* In addition, manipulations that disconnect mesencephalic structures from the neocortex also block EEG activation.[57] Thus, subanesthetic concentrations of thiopental may directly excite ascending systems leading to EEG activation. Alternatively, low thiopental concentrations may depress cortical electrical activity resulting in a disinhibition of lower brain stem systems that feedback to excite cortical neurons, resulting in EEG activation. Thus, thiopental-induced EEG activation *in vivo* may reflect indirect effects of thiopental on brainstem activating systems, but EEG states associated with deepening levels of anesthesia (δ, burst suppression, and isoelectric activity) appear to be caused, at least partly, by direct effects of thiopental on cortical neurons.

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