Pentobarbital Depressant Effects Are Independent of GABA Receptors in Auditory Thalamic Neurons

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Wan, Xiang and Ernest Puil. Pentobarbital depressant effects are independent of GABA receptors in auditory thalamic neurons. J Neurophysiol 88: 3067–3077, 2002; 10.1152/jn.00365.2002. Pentobarbital, a general anesthetic, has received extensive study for its ability to potentiate inhibition at GABA_A subtype of receptors for GABA. Using whole cell current-clamp techniques and bath applications, we determined the effects of pentobarbital and GABA receptor antagonists on the membrane properties and tonic or burst firing of medial geniculate neurons in thalamic slices. Pentobarbital (0.01–200 μM) induced depressant effects in 50 of 66 neurons (76%). Pentobarbital hyperpolarized neurons by a mean of 3 mV and decreased the number of action potentials in tonic firing, evoked by current pulse injection from near the resting potential. Pentobarbital also decreased burst firing or low threshold Ca^{2+}-spikes, evoked by current pulse injection into neurons at potentials hyperpolarized from rest. The blockade of tonic and burst firing, as well as low threshold Ca^{2+}-spikes, was surmountable by increasing the amplitude of input current. The GABA_A receptor antagonists, bicuculline (100 μM) and picrotoxinin (50–100 μM), did not block the depressant effects of pentobarbital (10 μM). The GABA_B receptor antagonist, saclofen (200 μM), and GABA_A receptor antagonist, (1,2,3,6-tetrahydropyridine-4-yl)methylphosphinate (10–50 μM), did not significantly alter the depressant effects. Pentobarbital produced excitatory effects (0.1–50 μM) on 11 neurons (17%) but had no effects on 5 neurons (7%). The excitation consisted of approximately 3 mV depolarization, increased tonic and burst firing and the rate of rise and amplitude of low threshold Ca^{2+} spikes. These effects were associated with a increase in input resistance. In contrast, the depressant effects of pentobarbital correlated to a decreased input resistance measured with hyperpolarizing current pulse injection (IC_{50} = 7.8 μM). Pentobarbital reduced Na^{+}-dependent rectification on depolarization and lowered the slope resistance over a wide voltage range. Tetrodotoxin eliminated both Na^{+}-dependent rectification and the pentobarbital-induced decrease in membrane resistance at depolarized voltages in two-thirds of the neurons. The pentobarbital-induced decrease in membrane resistance at voltages hyperpolarized from rest was not evident during co-application with Cs^{+}, known to block the hyperpolarization-activated rectifiers. In summary, the pentobarbital acted at low concentrations to depress thalamocortical neurons. The depression resulted from decreased rectification on depolarization, which no longer boosted potentials over threshold, and an increased conductance that shunted spike generation. The depressant effects of pentobarbital did not involve known types of GABA receptor interactions.

INTRODUCTION

General anesthetics interrupt communication between neurons by depressing their membrane excitability in several ways (reviewed by Knezevic and Puil 1997). A widely accepted mode of anesthetic action is an increased receptor sensitivity to inhibitory neurotransmitter. This is often viewed as an increased amplitude, and especially, a prolonged duration of inhibitory postsynaptic potentials (IPSPs) mediated by GABA (Nicol et al. 1975).

Barbiturate anesthetics, like pentobarbital, are well known for their ability to enhance the actions of GABA at the ionotropic GABA_A subclass of GABA receptors (reviewed by Mehta and Ticku 1999). This potentiation of transmitter action by pentobarbital, usually at <100 μM, results in greater synaptic inhibition due to an increased membrane conductance to Cl^- (Barker and Ransom 1978; Nicoll et al. 1975). Pentobarbital itself activates a distinct site on the GABA_A receptor, mimicking the actions of GABA (Barker and Ransom 1978; Mathers and Barker 1980). The GABA_A receptor antagonists, bicuculline and picrotoxinin, antagonize these direct actions which require higher concentrations of pentobarbital (Barker and Ransom 1978; Nicoll and Wojtowicz 1980; cf. Thompson et al. 1996).

Pentobarbital and other anesthetics depress membrane excitability by actions that are not sensitive to blockade with GABA_A receptor antagonists (Belelli et al. 1999; Sugiyama et al. 1992). Indeed, observations of greater GABAergic inhibition are not universal during anesthesia. Some anesthetics actually depress GABA_A-mediated IPSPs in hippocampal neurons (Fujiwara et al. 1988; Miu and Puil 1989), in addition to decreasing the postsynaptic responsiveness to excitatory transmitters (Puil and El-Beheiry 1990) and excitatory postsynaptic potentials (EPSPs; El-Beheiry and Puil 1989).

Pentobarbital has effects on the intrinsic membrane properties that depress the excitability of neurons. This depression occurs in the same range of concentrations that potentiate GABA action. A prominent effect of pentobarbital is to increase K^+-conductance that shunts the current required for excitation (Nicoll and Madison 1982; O’Beirne et al. 1987; Ries and Puil 1999; Sirois et al. 1998). Pentobarbital also inhibits Ca^{2+}-currents (French-Mullen et al. 1993; Werz and Macdonald 1985) and suppresses Ca^{2+}-dependent transmitter.
release (Weakly 1969). High concentrations of barbiturates depress Na\textsuperscript{+}-dependent action potentials and currents in axons (Blaustein 1968). Hence, anesthetics have membrane actions, unrelated to GABAergic inhibition that may contribute to, or account for anesthetic-induced unconsciousness.

Despite the major participation of thalamocortical neurons in conscious behavior, there are surprisingly few studies of anesthetic effects on the membrane properties of these neurons. For example, it is not known if anesthetics affect the Na\textsuperscript{+}-dependent rectification in thalamocortical neurons (Jahnsen and Lilinas 1984; Tennygeist et al. 1996), which modulates excitation in a voltage range near the thresholds for the Na\textsuperscript{+}-dependent action potential and low threshold Ca\textsuperscript{2+} spike (LTS; Parri and Crunelli 1998). Using iontophoretic drug application techniques, Sykes and Thomson (1989) have demonstrated that pentobarbital enhanced the actions of GABA and prolonged inhibitory postsynaptic potentials (IPSPs) in thalamocortical neurons. In our preliminary studies, pentobarbital had effects on the intrinsic properties and LTS of thalamocortical neurons that did not appear to involve GABA receptors (Puil et al. 1996). Extending these studies, we now report on the effects of pentobarbital and GABA receptor antagonists on the subthreshold and firing characteristics of neurons in in vitro thalamic slices.

For these investigations, we have chosen medial geniculate neurons that receive GABAergic inputs from dorsal thalamic and reticularis nuclei as well as from basal forebrain, basal ganglia, and brain stem (see Steriade et al. 1997). Receptors for GABA, particularly the GABA\textsubscript{A} and GABA\textsubscript{B} subtypes, are highly expressed in the medial geniculate nucleus (Bowery et al. 1987). Pentobarbital, a commonly used anesthetic in vivo studies of the auditory pathway, critically alters the distribution of response patterns to noise or tone bursts in medial geniculate neurons (Zurita et al. 1994). We have investigated pentobarbital’s effects, including a possible involvement of GABA receptors, in medial geniculate neurons of rats at the end of the second postnatal week.

METHODS

The experiments received approval from the Committee on Animal Care of The University of British Columbia. Using previously described procedures (Ries and Puil 1999; Tennygeist et al. 1996), coronal slices (approximately 300 \mu m) containing the ventral portion of the medial geniculate body (MGB) were prepared from brain tissue of anesthetized Sprague-Dawley rats (13–15 days). Most recordings were made in neurons from P14 rats. The slices were maintained in a holding chamber containing normal artificial cerebrospinal fluid (ACSF; 23–25°C) until needed for the experiment. Except for the initial preparation, the ACSF used for the experiments contained the following (in mM): 124 NaCl, 26 NaHCO\textsubscript{3}, 10 glucose, 4 KCl, 2 CaCl\textsubscript{2}, 2 MgCl\textsubscript{2}, and 1.25 KH\textsubscript{2}PO\textsubscript{4}. In the ACSF used for the slices, NaCl was replaced by 125 mM sucrose. The ACSF solutions were saturated with 95\% O\textsubscript{2}-5\% CO\textsubscript{2} and had a pH of 7.4.

Electrical recording

Whole cell patch-clamp recording was performed using an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA) in the current-clamp mode. The recording pipettes were drawn from borosilicate glass tubing with internal filament (WPI Instruments, Sarasota, FL), using a vertical puller (Narishige Instruments, Tokyo, Japan). The pipette solution contained the following (in mM): 140 K-glutamate, 5 KCl, 10 EGTA, 3 MgCl\textsubscript{2}, 10 HEPES, 2.8 or 3 disodium ATP, 0.3 monosodium GTP, and 1 CaCl\textsubscript{2}. The calculated E\textsubscript{K} was −55 mV and E\textsubscript{Na} was −84 mV. Just prior to electrical recording, ATP and GTP were added to the pipette solution. The pH was adjusted to 7.4 with 10\% gluconic acid. The electrode resistances ranged between 5 and 9 MΩ.

For recording, a slice was placed in a Perspex chamber that had a volume of 1.2 ml. A polypropylene mesh in the chamber immobilized the slice, which was perfused with oxygenated (95\% O\textsubscript{2}-5\% CO\textsubscript{2}) ACSF at a flow rate of 1 ml min\textsuperscript{−1}. The MGB was identified with the aid of differential interference contrast (DIC) microscopy (400× magnification). The measurements of drug effects were conducted on visually selected neurons at 23–25°C. We accepted neurons for further study if they had stable resting membrane potentials and responded to depolarizing current pulse injections with overshooting action potentials. The input resistance (R\textsubscript{i}) was determined from the hyperpolarizing voltage responses of usually approximately 5 mV, evoked by intracellular injections of current. The neurons were held at −66 mV for construction of current-voltage (I-V) relationships. We elicited tonic firing by injection of depolarizing current pulses into neurons held near the resting potential. As typical for neurons of dorsal thalamic nuclei, bursts of action potentials were elicited on injection of depolarizing pulses into neurons at hyperpolarized membrane potentials or on the rebound response to hyperpolarizing current pulses. Signals were low-pass filtered at 5 kHz and digitized at 10 kHz with a 16-bit data acquisition system (Axon Instruments), using pClamp 8 software running on a Pentium computer.

Drug application

Stock solutions of the drugs used in these experiments were prepared in distilled water or normal ACSF, diluted for immediate administration or frozen until just before the experiment. (±)-Pentobarbital was purchased from Durex Medical Surgical Products (Pickering, Ontario, Canada). Picrotoxin and TTX (0.6 μM), as well as separate batches of bicuculline methiodide, were purchased from Sigma-Aldrich Canada (Mississauga, Ontario, Canada). Muscimol was a generous gift of Dr. David Mathers. Scolofen was purchased from Precision Biochemicals (Vancouver, British Columbia, Canada) and (1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid (TPMPA) was purchased from RBI/Sigma.

Drugs were applied to the slices in the bath by superfusion. The results of several experiments, involving bath applications of ACSF containing 20 mM KCl and dyes showed that steady-state concentrations were reached at 4–6 min. In approximately one-third of the experiments, pentobarbital was applied in more than one concentration to each slice for concentration-response studies.

Data and statistical analyses

The data have been compensated for the junction potential between ACSF and the electrode solution. The junction potential of −11 mV was subtracted from all membrane potentials, e.g., a recorded resting potential of −55 mV corresponds to an actual potential of −66 mV (see Ries and Puil 1999; Zhang and Krnjevic 1993). The data were analyzed with pClamp 8 (Clampfit, Axon Instruments) or Prism GraphPad software (v. 2.0, San Diego, CA). Representative graphs were constructed using Prism software or CorelDraw software (v. 10, Ottawa, Ontario, Canada). We used Student’s t-tests for comparisons of two groups and testing for differences from a theoretical mean. Differences were considered significant when P < 0.05. Data are expressed as means ± SE, n = sample size, unless otherwise mentioned.

RESULTS

The data were obtained from 66 neurons, located in the ventral division of the MGB. At approximately 2 min before
drug application, the mean resting potential was $-66 \pm 2.4$ mV and mean $R_i$ was $236 \pm 26 \, \text{M} \Omega$ ($n = 20$).

Application of pentobarbital produced both depressant and excitatory responses on the firing and membrane properties of neurons, occasionally at 2–3 min, but typically at 4 min during 6-min applications. Depressant effects of pentobarbital (20 $\mu$M) occurred in 50 MGB neurons (76%). Pentobarbital at 10 and 100 nM, the lowest concentrations applied, did not have significant effects in five neurons (7%). In the remaining 11 neurons, pentobarbital (0.1–50 $\mu$M) increased excitability and firing.

**Depressant effects on tonic and burst firing**

Pentobarbital application decreased or eliminated tonic firing as well as burst firing and the associated low threshold Ca$^{2+}$-spike (LTS) evoked by current pulses. We determined the effects of pentobarbital on the tonic firing of four or five action potentials or LTS bursts of two or three action potentials evoked by a current pulse. Figure 1A shows the reduction in tonic firing due to pentobarbital application (20 $\mu$M) in a neuron held at $-66$ mV. Pentobarbital application decreased or eliminated bursts of action potentials, including the LTS in neurons held at $-86$ mV (Fig. 1B). These effects were reversible on terminating the application in all neurons. Complete recovery required 20–30 min in approximately 75% of all neurons.

Pentobarbital application (0.01–50 $\mu$M) produced an elevation in the amount of current required for evoking repetitive action potentials (Fig. 1) but only small changes (<10%) in action potential amplitude (Fig. 1D). The surmountable blockade was evident in neurons firing in tonic and burst modes. The tonic firing rate decreased with an increase in the pentobarbital concentration (Fig. 2A). The data were fitted with a sigmoidal function and showed an IC$_{50}$ at $7.2 \pm 0.7$ $\mu$M.

We investigated the pentobarbital-induced changes in the LTS on applying TTX to block voltage-dependent Na$^+$-conductances. After the blockade of action potentials with TTX, pentobarbital application (10 $\mu$M) significantly and reversibly decreased the maximal rate of rise ($dV/dt_{\max}$) of the LTS by 29% (from $2.2 \pm 0.1$ to $1.5 \pm 0.1$ mV/ms, $n = 5$). The LTS decreased gradually in amplitude during the application, and at approximately 4 min became a voltage response that likely reflected the passive membrane properties (Fig. 1C, middle). The LTS appeared shunted because an increase in the amplitude of injected current produced a return of the LTS (Fig. 1C, middle).

**Depressant effects on membrane properties**

**INPUT RESISTANCE AND MEMBRANE POTENTIAL.** Pentobarbital application produced changes in membrane properties that corresponded to the observed decrease in firing. Neurons that exhibited a decrease in evoked firing on 10 $\mu$M pentobarbital application showed a 28 ± 9% reduction in $R_i$ ($n = 5$), measured with hyperpolarizing pulses (Fig. 2C). Overall, pentobarbital application hyperpolarized neurons by a mean of 3 mV (range, 1–4 mV) which was not significantly different from the control ($n = 50$). Application of TTX did not significantly alter the resting potential and $R_i$ during the control period (Table 1, $n = 50$). Figure 2B shows the pooled data in a concentration-response relationship for the depressant effects of pentobarbital on $R_i$. The data, fitted with a sigmoidal function, showed an IC$_{50}$ at $7.8 \pm 0.5$ $\mu$M. The effects of 10 $\mu$M pentobarbital are summarized in Fig. 2C which shows input resistance data, measured with hyperpolarizing and depolarizing current pulses that displaced the membrane potential by approximately 8 mV. In cumulative concentration-response studies, pentobarbital application reversibly decreased $R_i$ in six of nine neurons, in association with decreased tonic and burst firing and in a concentration-dependent manner. In the remaining three neurons, however, pentobarbital produced a reversible, biphasic response. These were concentration-dependent (1–50 $\mu$M), consisting of 10–15 min of enhanced tonic or burst firing, a decreased $R_i$, and a subsequent approximately 5-min reduction in the evoked firing and $R_i$.

**SLOPE OF CURRENT-VOLTAGE RELATIONSHIP.** Before drug application, the relationships between current and voltage ($I$-$V$) for neurons exhibiting depressant responses were either, approximately linear, or S-shaped curves. In neurons with either $I$-$V$ relationship, pentobarbital application resulted in an approximately linear relationship with a reduced slope. As in Fig.
3A, we observed a reduction in the slope of the relationship over a membrane voltage range from −80 mV to threshold (approximately −50 mV) in 32 of 34 neurons that were administered pentobarbital in concentrations ≥10 μM. The reversal potential for pentobarbital action provided by the inter-

section of the control and pentobarbital curves (cf. Fig. 3A) was −72.1 ± 3.0 mV (n = 9).

The I-V relationship did not greatly change during application of TTX to 50 neurons at membrane potentials hyperpolarized from rest. During TTX application, pentobarbital retained an ability to decrease the slope of the I-V relationship over a −90 to −65 mV range (Fig. 3B). Application of TTX reduced the apparent input resistance at depolarized potentials, presumably by eliminating a voltage- Na+-dependent type of rectification in this quadrant (Fig. 3B; Jahnsen and Llinas 1984; Tennigkeit et al. 1996). In the neuron of Fig. 3B, a co-application of TTX with pentobarbital did not result in a decrease the slope of the I-V relationship from −65 to −40 mV. In this voltage range, there was a close superposition of the curves for TTX, alone and co-application of TTX with pentobarbital in 15 of 24 neurons. The reversal potential for pentobarbital action in the presence of TTX was −79.4 ± 2.7 mV (n = 15). In the remaining 9 of 24 neurons, however, there was a significant decrease of 18 ± 7% in the slope of the I-V relationship (Fig. 3C). Hence, an action of pentobarbital on TTX-insensitive forms of rectification (e.g., a K+-rectifier) was apparent in the top right quadrant in the nine neurons. In these neurons, the reversal potential for pentobarbital action was −74.3 ± 4.1 mV.

### TABLE 1. Membrane properties of neurons showing depressant or excitatory effects of pentobarbital

<table>
<thead>
<tr>
<th>Neurons Exhibiting Depressant Effects (n = 50, 76%)</th>
<th>Neurons Exhibiting Excitatory Effects (n = 11, 17%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control TTX</td>
<td>Control TTX</td>
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<tr>
<td><strong>R_i</strong> (MΩ)</td>
<td>204 ± 20</td>
</tr>
<tr>
<td><strong>R_{indep}</strong> (MΩ)</td>
<td>207 ± 25</td>
</tr>
<tr>
<td><strong>dV/dt</strong> of LTS</td>
<td>2.0 ± 0.3</td>
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<td>(mV/ms)</td>
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Values are expressed as mean ± SE. Number of pooled MGB neurons and percentages of total are given in parentheses. **R_i** was obtained from hyperpolarizing voltage responses (−5−8 mV) to current pulses. Input resistance in depolarizing direction (**R_{indep}**) was obtained from depolarizing voltage responses (−5−8 mV) to current pulses. **dV/dt** was obtained from just-threshold LTS. * Significantly different from control. P < 0.05.
mV (n = 9). There was no significant difference in the reversal potentials between these two groups of neurons.

External Cs⁺ blockade of depressant effects of pentobarbital

We co-applied 3 mM Cs⁺ and pentobarbital (20 μM) to investigate the effects of blockade of hyperpolarization-activated inward rectifiers (Pape 1996) on the depressant effects in five neurons. These experiments were performed during concomitant TTX application, to block voltage-dependent Na⁺ conductances. Prior to co-application with Cs⁺, pentobarbital induced a 29% decrease in Rᵢ (control, 214 ± 20 MΩ; pentobarbital, 151 ± 16 MΩ). Application of Cs⁺ alone increased Rᵢ by 71% (control, 214 ± 20 MΩ; Cs⁺, 368 ± 31 MΩ; Fig. 4A). The neurons depolarized by 8 ± 4 mV (n = 5) during the application. During co-application with Cs⁺, the depressant effects of pentobarbital were not apparent (Cs⁺, 368 ± 31 MΩ; Cs⁺ + pentobarbital, 365 ± 34 MΩ; Fig. 4A). Application of Cs⁺ also greatly increased the slope of I-V relationship in the same five neurons in a membrane potential range of −65 to −100 mV (Fig. 4B). Co-application of Cs⁺ and pentobarbital (20 μM) did not result in a further change in the slope (Fig. 4B).

Interactions with bicuculline, a GABAₐ receptor antagonist

We examined the interactions of a GABAₐ receptor antagonist, bicuculline (20–100 μM) to assess the possibility that pentobarbital produced the effects by activating GABAₐ receptors. In these experiments on five neurons, we first applied bicuculline (100 μM) for 4 min before co-applying pentobarbital (10 μM) and bicuculline (100 μM) for an additional 4 min. Bicuculline alone had an excitatory effect (cf. nucleus reticularis thalami neurons, Debarbieux et al. 1998), depolarizing neurons by 4–5 mV and producing a small leftward shift in the latency of evoked action potentials (Fig. 5A). Under these conditions, pentobarbital decreased both tonic (Fig. 5A) and burst firing, as well as the LTS during TTX-blockade of Na⁺ conductances. We also reversed the procedure, applying pentobarbital (10 μM) for 4 min, observing the depressant effects and a small hyperpolarization, and then co-applying bicuculline (100 μM) for an additional 4 min. In such cases,
Muscimol activation of GABA_A receptors

We used the GABA_A agonist, muscimol, to verify that the GABA_A receptors in the slices remained intact and were susceptible to antagonism by bicuculline. Application of muscimol (20 \( \mu M \)) to three neurons held at \(-66 \) mV produced a decrease in \( R_i \) from the control value of \( 252 \pm 30 \) to \( 90 \pm 17 \) \( M\Omega \) (\( n = 3 \)). Muscimol depolarized two neurons by approximately 1 mV and the third neuron by 4 mV. As expected for a \( Cl^- \) conductance increase, the depolarizing direction of these values is consistent with a relatively positive \( E_{Cl^-} \). Co-application of muscimol (20 \( \mu M \)) and bicuculline (50 \( \mu M \)) for 6 min did not result in significant changes in \( R_i \) (\( n = 3 \)). Approximately 10 min after termination of the co-application, an application of muscimol, alone, again produced fully reversible decreases in \( R_i \) in all three neurons. The depressant effects of muscimol that were susceptible to blockade by bicuculline implied that GABA_A receptors were functional in the preparation.  

Interactions with picrotoxinin, an antagonist of GABA_A-activated Cl^- channels

In view of the above results, we attempted to block pentobarbital actions by applying picrotoxinin (50 or 100 \( \mu M \)), which blocks a GABA_A-linked \( Cl^- \)-channel site, distinct from the site antagonized by bicuculline. We observed the effects of pentobarbital (10 \( \mu M \)) application during the first 4 min and then during an additional 4 min co-application with picrotoxinin (50 \( \mu M \); \( n = 3 \)). We also reversed the procedure in three additional neurons by applying picrotoxinin before pentobarbital. Picrotoxinin application to the six neurons of this study did not significantly alter the membrane potential and \( R_i \) (Fig. 5C). Pentobarbital, applied by either procedure, decreased both tonic and burst firing, as well as the LTS on TTX-blockade of voltage-dependent Na^+ conductances (Fig. 5B). Pentobarbital also increased the threshold current requirement for a LTS. After a 20-min recovery period from the co-application, a second application of pentobarbital alone produced depressant effects of approximately the same magnitude as in the initial control. Therefore picrotoxinin-blockade of the GABA_A receptor complex did not prevent the depressant effects of pentobarbital on thalamocortical neurons.

Interactions with saclofen, a GABA_B receptor antagonist

We applied saclofen, a GABA_B receptor antagonist, to assess a possible involvement of GABA_B receptors in pentobarbital actions. An initial application of pentobarbital (10 \( \mu M \)) during TTX-blockade of voltage-dependent Na^+ conductances eliminated the LTS and decreased \( R_i \) from 260 \pm 40 to 180 \pm 28 M\Omega \) (\( n = 5 \)). After recovery of the LTS and a subsequent application of saclofen (200 \( \mu M \)) for 4 min, followed by the co-application (4 min), pentobarbital still eliminated the LTS (Fig. 6A) and decreased \( R_i \) from 256 \pm 35 to 175 \pm 30 M\Omega \) (Fig. 6C; \( n = 5 \)). Despite the co-application with a saclofen concentration that antagonizes GABA_B responses in MGB neurons (Peruzzi et al. 1997), pentobarbital produced depressant responses.

Interactions with a GABA_C receptor antagonist

We examined the interactions of pentobarbital and TPMPA, a GABA_C receptor antagonist (Ragozzino et al. 1996), to assess a possible contribution of GABA_C receptor activation to the depressant effects. We applied the same procedure used for saclofen, for TPMPA. Before TPMPA, pentobarbital application (10 \( \mu M \)) abolished the LTS and decreased \( R_i \) by 30\%, from 244 \pm 30 to 170 \pm 24 M\Omega \) (\( n = 4 \)). An 8-min application of TPMPA, in concentrations that ranged between 10 and 50 \( \mu M \), did not block the inhibition of the tonic and burst firing (Fig. 6B), the LTS, or the decrease in \( R_i \) induced by co-applied pentobarbital. During co-application of TPMPA and pentobarbital (10 \( \mu M \)), \( R_i \) decreased 31\%, from 238 \pm 40 to 168 \pm 33 M\Omega \) (Fig. 6C; \( n = 4 \)). In summary, pharmacological blockade of GABA_C receptors produced no antagonism of the depressant effects of pentobarbital on MGB thalamocortical neurons.

Co-application with GABA_A, GABA_B, and GABA_C antagonists

We co-applied pentobarbital with the GABA_A receptor antagonist, picrotoxinin (50 \( \mu M \)), GABA_B receptor antagonist, saclofen (200 \( \mu M \)), and GABA_C receptor antagonist, TPMPA (20 \( \mu M \)), to verify that known GABA receptors did not mediate the pentobarbital-induced depression. Before applying the three antagonists, application of pentobarbital (20 \( \mu M \)) induced a 33 \pm 7\% decrease in \( R_i \) (\( n = 3 \)). During co-application...
with the antagonists, pentobarbital decreased $R_i$ by 31/6% ($n = 3$). In summary, combined blockade of $\mathrm{GABA}_A$, $\mathrm{GABA}_B$, and $\mathrm{GABA}_C$ receptors did not significantly alter the ability of pentobarbital to decrease $R_i$.

Excitatory effects of pentobarbital

In 11 of the 66 neurons, application of pentobarbital (0.1–50 µM) produced excitatory effects. The number of excited neurons was too small for a systematic comparison with the 50 neurons that exhibited depressant responses to pentobarbital application. The excitation consisted of an increased action potential discharge in the tonic and burst patterns evoked by current pulses (Fig. 7A). In 3 of 11 neurons, there was a greater number of action potentials following an evoked burst. Pentobarbital application decreased the current requirement for evoking a LTS and increased its rate of rise and amplitude. A blockade of $\mathrm{Na}^+$-conductances with TTX did not alter the effects on the LTS (Fig. 7A). The excitatory effects were reversible and included a small depolarization and increased $R_i$, measured with hyperpolarizing pulses. At 20 µM, pentobarbital produced $3 \pm 1$ mV depolarization and a $22 \pm 4\%$ increase in $R_i$ ($n = 3$). Pentobarbital application also increased the slope resistance in four neurons at subthreshold potential values (Fig. 7C). The higher resistance reduced the threshold current requirement and increased the tonic firing. Figure 7B shows the concentration-response relationship for the 11 neurons that showed increases in $R_i$ due to pentobarbital application. Recovery was complete in 9 of 11 neurons at 20–25 min after discontinuing the application.

The pentobarbital-induced increase in input and slope resistances, measured with depolarizing current pulses, was not evident after TTX blockade of $\mathrm{Na}^+$-dependent action potentials and rectification. Application of TTX, alone, produced a $32 \pm 5\%$ decrease in input resistance, measured from 5–8 mV depolarizing responses to current pulses ($n = 7$). This decrease was attributable to a TTX blockade of a persistent $\mathrm{Na}^+$-current (Parri and Crunelli 1998). Prior to TTX blockade, pentobarbital evoked a $30 \pm 3\%$ increase in input resistance, measured with depolarizing current pulses ($n = 4$). During TTX blockade, pentobarbital had no significant effects on resistance measured with depolarizing pulses ($n = 7$). In contrast, co-application with TTX did not significantly affect the pentobarbital-induced increase in $R_i$ ($26 \pm 6\%; n = 7$) or slope resistance, measured
block the pentobarbital-induced decreases in tonic and burst firing. These agents, which interact with distinct sites on the GABA_\text{A} receptor complex, did not affect the accompanying increase in input conductance which is similar to the findings in frog spinal motoneurons (Nicoll and Madison 1982). Bicuculline and picrotoxinin also did not antagonize pentobarbital’s ability to decrease the low threshold T-type Ca^{2+} spikes in thalamocortical neurons. Interestingly, these antagonists do not block the pentobarbital suppression of L-type Ca^{2+} plateau potentials in turtle spinal motoneurons (Guertin and Hounsgaard 1999). In neocortical neurons of P0–P1 rats, bicuculline antagonizes the depressant effects of GABA_\text{A} receptor agonists and some general anesthetics, but not pentobarbital (Antkowiak 1999). In embryonic neurons of human dorsal root ganglia, GABA_\text{A} antagonists suppress the Cl\textsuperscript{−} current induced by GABA, but not alphaxalone, an anesthetic neurosteroid (Vallelyev et al. 1999a,b). Hence, a resistance to GABA receptor antagonists is evident from studies of the depressant effects of anesthetics on both adult and immature neurons.

We observed that pentobarbital application evoked a depression that was insensitive to GABA_\text{A} antagonists in MGB neurons of the ventral division from rats aged P14. These neurons cease to show discernible development changes in their morphological and electrical membrane properties after P13 (Tennigkeit et al. 1998a). The insensitivity to GABA_\text{A} antagonists is probably not due to specialization of immature brain because auditory transmission, as evident in the behavior of young rats after postnatal day 13, is similar to that of the adult (Ehret 1983; Rubel 1978). During development, the subunit composition and expression of GABA receptors undergo changes. However, there is high expression of thalamic GABA receptors at postnatal day 14 (Laurie et al. 1992; Okada et al. 2000). The principal GABA_\text{A} subunit transcripts, \(\alpha_1\), \(\alpha_3\), \(\beta_2\), and \(\delta\) mRNA, reach adult levels in the thalamus of rats by P12 (Laurie et al. 1992; Wisden et al. 1992). Attempts to define a receptor subunit composition for sites of anesthetic action have shown that GABA action requires the presence of the \(\alpha\) subunit whereas the \(\beta\) subunit enhances the sensitivity of GABA_\text{A} receptors to pentobarbital (Cestari et al. 1996; Harris et al. 1995; Thompson et al. 1996). Hence, medial geniculate neurons at the end of the second postnatal week should have functional GABA receptors and a sensitivity to pentobarbital.

We have demonstrated that GABA_\text{A} receptors were functional in our slice preparations. Application of muscimol, a GABA_\text{A} receptor agonist, produced a large, reversible increase in input conductance which was completely blocked by bicuculline. Hence, it is unlikely that the insensitivity of pentobarbital’s effects to GABA_\text{A} antagonists resides in a dysfunctional organization of the GABA_\text{A} receptor (Cherubini and Conti 2001; Thompson et al. 1996). The insensitivity to GABA_\text{A} antagonists and low IC_{50} distinguish the pentobarbital depression in thalamocortical neurons from the “direct or GABA-mimetic” effects produced by at least 10-fold higher concentrations of pentobarbital (Barker and Ransom 1978; Nicoll and Wojtowicz 1980; cf. Thompson et al. 1996). Furthermore, we observed that GABA_\text{B} and GABA_\text{C} receptor antagonists, and even combined application of GABA_\text{A}, GABA_\text{B}, and GABA_\text{C} antagonists, did not significantly alter the depression produced by pentobarbital. The insensitivity of pentobarbital’s effects on thalamocortical neurons to antagonists of known subtypes of...
Pentobarbital blockade of firing

Pentobarbital blocked the tonic and burst firing of Na\(^{+}\)-dependent action potentials in thalamocortical neurons by a mechanism that involved a decrease in Na\(^{+}\)-dependent inward rectification on depolarization to threshold. Normally, these neurons inwardly rectify in a range between the resting potential and threshold (approximately −50 mV; Jahnson and Linas 1984; Tennykeit et al. 1996), due to a persistent Na\(^{+}\) current that activates at potentials as low as −75 mV (Parri and Cruneli 1998). In the present studies, pentobarbital application attenuated depolarizing voltage responses and Na\(^{+}\)-dependent rectification, producing a slower firing rate. These effects are similar to those evoked by phenytoin in cortical neurons (Lamp et al. 1998). TTX eliminated both Na\(^{+}\)-dependent rectification and the pentobarbital-induced decrease in membrane resistance at depolarized voltages in a majority of thalamocortical neurons. An increase in the input current evoked Na\(^{+}\)-dependent action potentials during pentobarbital application. The rate of rise of action potentials did not decrease substantially during the pentobarbital blockade of Na\(^{+}\)-dependent rectification. This implies that pentobarbital application may produce effects similar to TTX application at low concentrations, i.e., a greater block of the persistent Na\(^{+}\) current rather than the transient Na\(^{+}\) current which underlies the action potential (cf. Blaustein 1968; Tennykeit et al. 1998).

In the present studies, the depressant effects of pentobarbital on the input and slope resistances in the hyperpolarizing quadrant may have involved an increase in leak and hyperpolarization-activated rectifier conductances that were sensitive to Cs\(^{+}\) blockade. We observed a reversal potential for pentobarbital action (−72 mV) that was consistent with Na\(^{+}\) and K\(^{+}\) conductance involvement. However, the exact type of conductance increase initiated by pentobarbital remains unclear. We ob-
served that pentobarbital reduced input resistance and slope resistance in all neurons at hyperpolarized potentials. These changes were not evident during co-application of pentobarbital with Cs\(^+\), which blocks the hyperpolarization-activated inward rectifiers, \(I_R\) and \(I_{Ks}\) (Pape 1996). Application of Cs\(^+\) alone greatly elevated the input resistance and depolarized the neurons, presumably due to decreased leak and rectifier conductances. Despite the uncertainty about the conductance type, the increased conductance due to pentobarbital application (cf. Gibbons et al. 1996) would shunt the current required for action potential generation.

**Excitatory effects**

In 17% of neurons, pentobarbital application enhanced firing of action potentials and LTSs. These concentration-dependent effects resulted from increases in input resistance and inward rectification on depolarization in a 10–15 mV range, sub-threshold to action potential genesis. Application of TTX eliminated this rectification but not the pentobarbital-induced effects on the input resistance measured at hyperpolarized potentials. The higher input resistance and greater inward rectification on depolarization can account for the observed excitation and increased LTSs in thalamocortical neurons.

**Differences in neuron groups exhibiting depressant and excitatory effects**

It is unclear why pentobarbital induced opposite effects in different thalamocortical neurons. In a few neurons, we observed an excitation at low concentrations, followed by a depression at high concentrations. Neurons that were depressed by pentobarbital tended to have lower input resistances, less inward rectification on depolarization, and lower rate of rise of the LTS than neurons that were excited by pentobarbital (cf. Table 1). The excitatory effects induced by pentobarbital may relate to decreased inhibitory transmitter release (Collins 1981), increased excitatory transmitter release (Rohde and Harris 1983), or to the existence of distinct subpopulations of thalamocortical neurons (Turner et al. 1997).

**Significance of pentobarbital’s effects**

Pentobarbital produced mostly depressant and occasionally excitatory effects in thalamocortical neurons in vitro. The depression and excitation may relate to a spectrum of effects observed in vivo. The administration of pentobarbital for sedation and anesthesia would result in cerebrospinal fluid concentrations between 10 and 50 \(\mu\)M (Sato et al. 1995), which are similar to the concentrations used in the present studies. These concentrations would pertain to excitement, sedation, and unconsciousness.

The pentobarbital-induced excitation and depression of MGB neurons are relevant to auditory processing during sleep and consciousness. The pentobarbital-evoked decreases in tonic and burst firing would disrupt auditory communication in the cortico-thalamocortical system. The changes observed here in neurons of this system could affect the discharge properties and distribution of response patterns to noise, tone bursts and oscillatory signals, as observed during pentobarbital anesthesia in vivo (Zurita et al. 1994).

In summary, pentobarbital at low concentrations depressed a majority of auditory thalamic neurons. Pentobarbital markedly depressed their abilities to fire action potentials in tonic patterns and low threshold Ca\(^{2+}\)-spikes in the burst mode. The induced depression resulted from decreased Na\(^+\)-dependent rectification on depolarization that no longer boosted potentials above threshold and an increased membrane conductance that shunted spike generation. The depressant actions do not involve known types of GABA receptor interactions and warrant further study to elucidate the molecular targets for the novel effects of pentobarbital.

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