ly temporary deposition rather than an accumulation of enzyme over 2 or 3 days, it is unknown for how long the immunoreactive enzyme has been present in the observed locations.

These data suggest that collagenase is released by the long dendritic processes (Fig. 2), and that the large number of dense bodies found in these extensions (Fig. 1) may be related to enzyme storage or release. Immuno-ultrastructural studies may help to elucidate the mechanism of collagenase secretion or exocytosis in these cytoplasmic processes, whether the perinuclear region of cells demonstrating FITC-fluorescence represents Golgi apparatus, and how the enzyme is transported to the site of secretion.

It is not known whether the dendritic cell observed in vitro in monolayer cultures has the same morphology in vivo, or what the origin or distribution of this cell is in rheumatoid synovial tissue. The dendritic cells may be synovial cells transformed in morphology and function by components of chronic inflammation, much as bone cells may be transformed in vitro from a spherical to a stellate appearance by certain hormones and cyclic nucleotides (*14*). Since we now know that collagenase is produced at sites of cartilage resorption in vivo (*5*) and that the dendritic cell has the ability to produce large quantities of this enzyme in vitro, further studies on the role of this cell in the pathophysiology of the rheumatoid joint are essential.

DAVID E. WOOLLEY
University Department of Medicine, University Hospital of South Manchester, West Didsbury, Manchester M20 8LR, England
EDWARD D. HARRIS, JR.
CARLO L. MAINARDI
CONSTANCE E. BRINCKERHOFF
Department of Medicine, Dartmouth-Hitchcock Medical Center, Hanover, New Hampshire 03755

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Different Actions of Anticonvulsant and Anesthetic Barbiturates Revealed by Use of Cultured Mammalian Neurons

Abstract. *Barbiturate anesthetics, but not anticonvulsants, abolish the spontaneous activity of cultured spinal cord neurons; directly increase membrane conductance, an effect which is suppressed by the γ-aminobutyric acid (GABA) antagonists picrotoxin and penicillin; and are more potent than anticonvulsants in augmenting GABA and depressing glutamate responses. Barbiturate anticonvulsants abolish picrotoxin-induced convulsive activity. These results indicate qualitative and quantitative differences between anesthetic and anticonvulsant barbiturates, which may explain their different clinical effects.*

Barbiturates are used therapeutically for their varied pharmacological effects. Phenobarbital is an effective anticonvulsant at clinical doses that produce minimal sedation, while pentobarbital is commonly used as an anesthetic because of its dominant sedative action. Such disparate pharmacological effects may reflect different mechanisms for attenuating central nervous system (CNS) excitability, although such a comparative study has not been performed. Pentobarbital has multiple effects, including (i) enhanced presynaptic inhibition of sensory afferents (*1*), possibly mediated by γ-aminobutyric acid (GABA) (*2*); (ii) depression of transmitter release at sensory afferent synapses into the CNS (*3*); (iii) enhanced transmitter release at peripheral neuromuscular junctions (*4*); (iv) depression of excitatory synaptic transmission at a variety of peripheral and central synapses (*5–7*); (v) enhancement of GABA-mediated postsynaptic inhibition (*7–10*); and (vi) direct effects on sensory afferents and spinal cord neurons (*1, 7, 9, 10*) that are abolished by the GABA antagonist picrotoxin (*1, 9*). Thus, barbiturate anesthesia is thought to result from the combined effect of enhanced inhibition and diminished excitation, producing a net decrease in neuronal excitability.

We have compared the actions of anesthetic and anticonvulsant barbiturates using a mammalian tissue culture system. We have found that (i) anesthetics, but not anticonvulsants, abolish all spontaneous synaptic activity at therapeutic concentrations, and anticonvulsants abolish picrotoxin-induced paroxysmal events; (ii) anesthetics, but not anticonvulsants, directly increase membrane conductance, an effect which can be antagonized by the GABA-specific antagonists picrotoxin and penicillin (*11, 12*); (iii) anesthetics, but not anticonvulsants, substantially prolong GABA responses; and (iv) both classes of barbiturates enhance postsynaptic GABA responses and antagonize postsynaptic glutamate responses, although anesthetics are two to three times more potent than anticonvulsants. These results indicate qualitative and quantitative differences in the pharmacological actions of anticonvulsant and anesthetic barbiturates, which may explain their different clinical effects.

Spinal cords were dissected from 12-to 14-day-old mouse embryos, mechanically dissociated, and grown in tissue culture for 5 to 13 weeks before electrophysiological study (*13*). Recordings were made on a modified stage of an inverted phase microscope. Large, multipolar spinal cord neurons were penetrated under direct vision with glass micropipettes (25 to 50 megohms filled with 4*M* potassium acetate or 3*M* KCl. The legends to Figs. 1 and 2 contain additional details of methodology.

Spontaneous activity in these cultures (Fig. 1A1) was characterized by abundant excitatory and inhibitory postsynaptic potentials, random firing of action potentials with varying frequency, and occasional short duration (< 0.5 sec-

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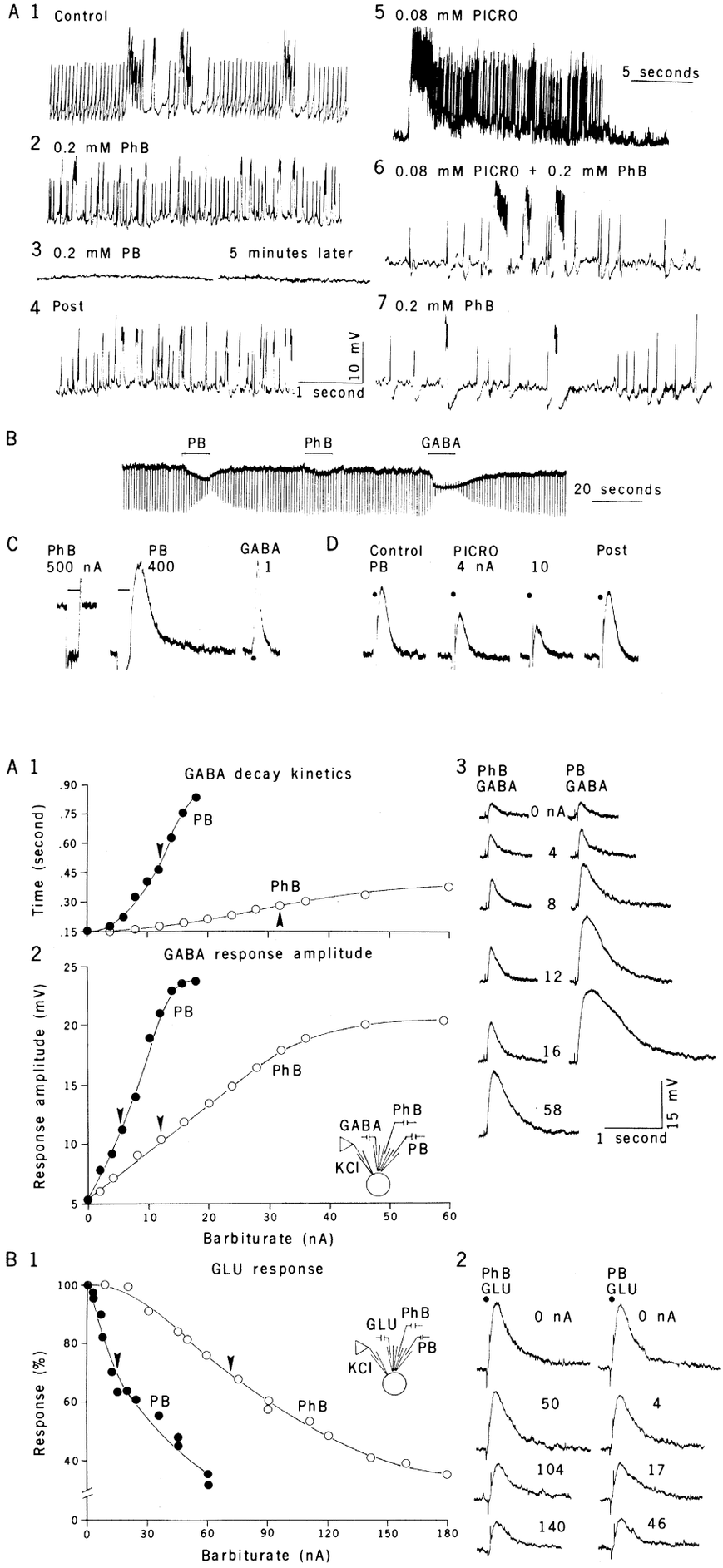

ond) bursts of action potentials. Addition of 0.2 m*M* phenobarital produced little alteration in spontaneous activity (Fig. 1A2), with slightly increased neuronal firing rate and decreased frequency of short bursts. Addition of the same concentration of the anesthetic pentobarbital produced an electrically silent state with no discernible synaptic activity (Fig. 1A3), an effect that was rapidly reversible (Fig. 1A4). Application of 0.08 m*M* picrotoxin

Fig. 1. (A) Barbiturate action on spontaneous activity recorded intracellularly from the same neuron over a 2-hour period. Phenobarbital (*PhB*) (0.2 m*M*), pentobarbital (*PB*) (0.2 m*M*), and picrotoxin (*PICRO*) (0.08 m*M*) were added to normal growth medium and perfused into the culture dish in volumes sufficient to exchange the medium four times. Records are from a pen recorder, and therefore the action potentials are attenuated. The 10 mV × 1 second bars apply throughout except in (A5) and (B) (time is 5 and 20 seconds, respectively). (B) Effect of barbiturates and GABA on membrane potential and conductance. Intracellular recordings were made with micropipettes filled with 4*M* potassium acetate, and iontophoresis was achieved with high-resistance (50 to 80 megohms) theta micropipettes filled with PB (0.2*M*, *p*H 9.0), PhB (0.2*M*, *p*H 9.0), and GABA (0.5*M*, *p*H 3.5). Membrane conductance was assessed by recording the voltage response to repeated current pulses (40 msec) delivered to the neuron using a standard bridge technique. Application of PB (300 nA) and GABA (50 nA), but not PhB (300 nA), produce membrane hyperpolarization and an increase in membrane conductance. Membrane potential was −50 mV. The 10-mV bar applies, but the time bar represents 20 seconds. (C) Direct effect of iontophoresed PB. The response to PB and GABA is reversed by use of recording micropipettes filled with 3*M* KCl. Membrane potential in (C) and (D) was −90 mV. (D) The PB response (200 nA) was rapidly and reversibly antagonized by concurrent iontophoresis of picrotoxin (10 m*M*, *p*H 4.5) (KCl recording micropipettes). Fig. 2. Barbiturate modulation of GABA and glutamate responses elicited from cultured mammalian spinal cord neurons. Intracellular recordings were made with micropipettes filled with 3*M* KCl, and iontophoresis was achieved using GABA, glutamate, PB, and PhB. Amino acids were passed with 50-msec pulses of appropriate polarity while barbiturates were applied with steady anionic current. The schematic insets in (A2) and (B1) illustrate the relationship between the neuronal cell body and recording and ionophoretic micropipettes. (A) Both PB and PhB augmented the GABA response (A3). PB produced the effect at much lower iontophoretic currents (A2) and also produced a pronounced increase in the decay time of the response (A1). (B) Both barbiturates antagonized the glutamate response (B2) without significant alteration in the response kinetics. PB was effective at lower iontophoretic currents (B1). Arrowheads indicate iontophoretic currents producing half-maximal effects on the various parameters. The data plotted in (A1), (A2), and (B1) are derived from the experiments illustrated in (A3) and (B2), respectively, and are representative of responses obtained with 15 different neurons.

led to the development of paroxysmal depolarizing events (PDE), characterized by random, abrupt depolarizations associated with bursts of action potentials (Fig. 1A5). These events were similar to the paroxysmal depolarizing shifts recorded in cortical neurons exposed to convulsant agents (*14*). Addition of 0.2 m*M* phenobarbital to the picrotoxin medium (Fig. 1A6) abolished the PDE and restored a spontaneous activity more characteristic of control recordings, or that with phenobarbital alone (Fig. 1A7). Thus, at identical concentrations, pentobarbital abolished spontaneous activity, and phenobarbital attenuated the picrotoxin-produced convulsive effect with minimal alteration in baseline spontaneous activity. Clinically, at this concentration, pentobarbital would produce anesthesia while phenobarbital would not; phenobarbital would be in therapeutic range as an anticonvulsant.

To determine how these structurally similar barbiturates produced such dissimilar effects on spontaneous neuronal activity, we investigated their effects on neuronal membrane properties and on responses to the iontophoretically applied GABA and glutamate. When applied from a pipette containing a 0.2*M* concentration with currents up to 500 nA, phenobarbital produced no response in eight of nine cells tested (Fig. 1B), and a small response in the remaining cell. Pentobarbital applied from a 0.2*M* solution increased membrane conductance and altered membrane potential in a reversible, dose-dependent manner (Fig. 1B). Use of 3*M* filled recording micropipettes reversed the direct pentobarbital and the GABA responses (Fig. 1C); this would be expected for a chloride-mediated conductance change. The direct PB response was rapidly and reversibly antagonized in a dose-dependent fashion by both picrotoxin (Fig. 1D) and penicillin (unpublished observations). Picrotoxin and penicillin do not affect neuronal membrane properties at doses that produce the specific antagonists of GABA-mediated postsynaptic inhibition in cultured murine spinal cord neurons (*12*). Thus, pentobarbital is "GABA-mimetic" when applied directly, that is, it appears to interact with GABA receptors to produce an increase in neuronal chloride conductance.

Pentobarbital and phenobarbital both augmented the amplitude of the GABA response (Fig. 2A3), an effect that required less iontophoretic current with pentobarbital than phenobarbital in 30 cells (Fig. 2A2). If similar transport numbers are assumed for both, these data would suggest that pentobarbital was more potent. In addition, pentobarbital slowed the kinetics of the GABA response in a dose-dependent manner, while phenobarbital did not (Fig. 2A1). Enhancement of the GABA response at low pentobarbital iontophoretic currents occurred without the direct pentobarbital effect; at higher currents, the direct effect was generally produced. Phenobarbital produced no such direct effect over similar ranges of GABA augmentation in eight trials. Thus, it would appear that the concentration of pentobarbital at which GABA augmentation occurs overlaps the concentration that produces the direct membrane effect; phenobarbital enhances GABA at concentrations which produce no direct effects. Both barbiturates antagonized the glutamate response without significant alteration in the response kinetics, pentobarbital again being two to three times more potent. Thus, (i) only pentobarbital is GABA-mimetic and appears to activate postsynaptic GABA receptors at the doses employed in this study; (ii) pentobarbital both augments and prolongs GABA responses while phenobarbital only augments them; (iii) both compounds antagonize glutamate responses; and (iv) in all cases, pentobarbital appears to be the more potent. Similar differences in pharmacological effects have been observed with the anesthetic secobarbital and the anticonvulsant mephobarbital. The ability of the anticonvulsant barbiturates to increase GABA-ergic inhibition and to decrease glutaminergic excitation might then form the basis for anticonvulsant action, while the addition of widespread inhibition produced directly by pentobarbital to the modulation of synaptic activity could form the basis for anesthetic action.

The mechanism of GABA augmentation by the barbiturates is unclear, but it is likely to involve a receptor interaction, since other neutral amino acids utilizing the same conductance mechanisms (*15*) are not affected. Additionally, preliminary analysis of Lineweaver-Burk plots suggests that the barbiturates increase the apparent affinity of the receptor for GABA (unpublished observations). The prolongation of the GABA response by pentobarbital could be produced by diminished presynaptic uptake of GABA, increased duration of the unitary conductance change produced by GABA, or decreased cooperativity of the GABA receptor. Pentobarbital does not alter GABA uptake by synaptosomes (*16*), making altered GABA uptake an unlikely cause of the prolongation. Further analysis of GABA dose-response data is necessary to determine which of these mechanisms is involved. In addition to their postsynaptic effects, it is likely that phenobarbital modulates presynaptic events as does pentobarbital (*1, 3*), and that such an effect also contributes to the therapeutic action of phenobarbital.

Our present results may provide the cellular basis for the different pharmacological actions of anticonvulsant and anesthetic barbiturates. They suggest that the distinction between an anesthetic and anticonvulsant barbiturate depends on the concentration at which amino acid modulation and GABA-mimetic activities occur. If both effects are present at similar concentrations, the agent would have primarily anesthetic properties, whereas if the threshold for modulatory activity is lower, the agent would be an effective anticonvulsant with sedation a feature only at toxic doses.

ROBERT L. MACDONALD
Laboratory of Developmental Neurobiology, National Institute of Child Health and Human Development, Bethesda, Maryland 20014, and *Department of Neurology, University of Virginia, Charlottesville 22903*

JEFFERY L. BARKER
Laboratory of Neurophysiology, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Maryland 20014

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