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Pentobarbital: Stereospecific Actions of (+) and (−) Isomers Revealed on Cultured Mammalian Neurons

Abstract. *Stereoisomers of the barbiturate anesthetic pentobarbital were applied to mouse spinal neurons growing in tissue culture. Intracellular recordings of neuronal membrane properties revealed that the (+) and (−) isomers caused direct changes in membrane potential and conductance on some but not all of the cells tested. The action of the (+) isomer was predominantly excitatory, whereas the (−) isomer produced predominantly inhibitory responses. The (−) isomer was considerably more effective in potentiating inhibitory responses to the transmitter gamma-aminobutyric acid. The results show that pentobarbital has multiple effects on neuronal excitability and demonstrate the presence of stereospecific sites of barbiturate action on central neurons.*

Racemic mixtures of barbiturates are used clinically as hypnotics and general anesthetics. However, subhypnotic doses are sometimes associated with stimulation of the central nervous system (CNS) (*1*) and preanesthetic excitation (*2*) as well as a hyperalgesic state (*3*). The (+) isomer of the anesthetic barbiturate pentobarbital (PB) causes a transient period of extreme hyperexcitability before depressing excitability in the CNS, whereas the (−) isomer produces a relatively smooth and progressively deeper hypnotic state (*4*). Similar differences in effects on excitability have been observed with other asymmetrically substituted barbiturates that can be resolved into stereoisomers (*2, 5*). At the cellular level racemic mixtures of clinically important barbiturates have multiple depressant actions on the excitability of vertebrate central and peripheral neurons in vivo (*6*) and in vitro (*7-9*). We have studied the cellular mechanisms underlying the stimulant and depressant effects of the barbiturate isomers by applying them to cultured mouse spinal neurons and report here evidence of cellular actions that correlate well with the clinical effects of the isomers. The results provide electrophysiological evidence for stereoselective requirements for the pharmacological effects of PB.

Neurons were dissociated from embryonic spinal cords and grown in tissue culture according to methods described (*10, 11*). Using conventional recording techniques we obtained intracellular microelectrode recordings from spinal cord cells on the modified stage of an inverted phase microscope. Prior to the experiment, growth medium was replaced with recording medium consisting of Hanks solution buffered to *p*H 7.4 with 25 m*M* Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid]. We added 10 m*M* $MgCl_2$ in many experiments to suppress synaptic activity and allow clearer examination of membrane events. The (+) and (−) isomers of PB were dissolved in the recording medium and then applied to individual neurons by pressure from pipettes containing 1 to 200 μM barbiturate that were positioned within 50 μm of the cell surface. The final concentration of drug bathing the neuron is unknown but is likely to be close to that present in the pipettes. Gamma-ammobutyric acid (GABA) (1*M*; *p*H 3.5) (Sigma, St. Louis), was applied by iontophoresis from pipettes positioned within 5 μm of the cell surface.

In 62 percent of the cells studied (18 of 29 neurons) brief application of (+)-PB depolarized the membrane potential in a dose-dependent, reversible manner. The depolarizing response was usually prolonged and outlasted the drug application period (Fig. 1A). The amplitude of the response increased as the membrane was hyperpolarized with the "null potential" for the response extrapolating to a potential more depolarized than the threshold for action potential generation. In ten cells (+)-PB also evoked excitatory synaptic activity, as evidenced by the appearance of synaptic potentials. These are shown superimposed on the depolarizing excitatory responses in Fig. 1, A_2. The presence of increased Mg^{2+} was clearly not sufficient to block the evoked synaptic activity. The evoked synaptic activity could result from either direct stimulation of transmitter release from innervating terminals or from indirect stimulation of transmitter release due to excitation of neurons presynaptic to the recorded cell. Sustained application of 100 to 200 μM (+)-PB produced complex membrane effects in four cells. The responses consisted of a transient depolarization followed by a hyperpolarization and increase in membrane conductance that persisted for the duration of the application (Fig. 1, B_3). After termination of the (+)-PB application, the membrane potential first depolarized and then repolarized to the control level. The drug did not produce any effect on eight cells whose membrane properties resembled those of cells responsive to (+)-PB. Since the cultures contain a heterogenous mixture of cells, it is likely that different types of neurons with apparently similar electrical properties may display different drug sensitivities.

The (−) isomer of PB depressed excitability in 11 of 30 cells studied, causing an increase in membrane potential and conductance (Fig. 1A). The null potential of the inhibitory response shifted in a depolarizing direction when KCl recording pipettes were used and was similar to that of inhibitory GABA responses. These results suggest that (−)-PB, like GABA (*11*) and racemic PB (*9*) may activate a Cl^- conductance, although further research is required to prove this. When 100 μM (+)-PB and (−)-PB were applied simultaneously, (−)-PB markedly attenuated excitatory responses to (+)-PB and blocked (+)-PB–evoked transmitter release (Fig. 1, A_2). Since 100 μM approximates the concentration of racemic PB necessary to induce general anesthesia (*12*), the depressant effects of the (−) isomer correlate well with the depression of the CNS seen clinically. Whether (−)-PB antagonizes (+)-PB excitation directly at excitatory sites or indirectly through its effective shunting of membrane resistance remains to be established. In three of the cells responding to (−)-PB a transient weak excitatory response was observed followed by

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the inhibitory effect just described (Fig. 1B).

Another action of racemic PB observed in a variety of central and peripheral neuronal preparations is a potentiation of responses to the putative inhibitory amino acid transmitter GABA (*6–9*). We examined the stereospecific requirements for this action by studying the effects of 100 μM (+)-PB and (−)-PB on GABA responses elicited on 26 cells. We found that (−)-PB greatly potentiated GABA-induced conductance on 18 cells (Fig. 2); (+)-PB potentiated GABA responses to the same degree on three cells, to a much lesser degree on three other cells (Fig. 2) and not at all on 20 other cells. As shown in Fig. 2, neither (+)-PB nor (−)-PB directly altered membrane properties in the cell but both enhanced the conductance induced by GABA. The (−)-PB enhanced the GABA-induced conductance 7.7-fold, whereas (+)-PB increased it only 1.4-fold. The time constant of decay of the GABA-induced conductance was increased 2.3-fold by (−)-PB and 1.2-fold by (+)-PB. These observations suggest that there are also stereospecific requirements for the interaction between PB and GABA receptor-coupled conductance mechanisms.

Thus the stereoisomers of PB have distinctly different actions on cultured spinal neurons. Although sustained applications of 100 μM (+)-PB can activate inhibitory conductance mechanisms on some cells (Fig. 1, B_3) and brief application of 100 μM (−)-PB can transiently excite a minority of the cells tested (Fig. 1B), the primary action of the (+) isomer is excitatory and that of the (−) isomer is inhibitory. Since excitatory and inhibitory responses were not always present on the same neuron, the sites of barbiturate action appear to be relatively independent. The isomers may interact with each other on those cells where

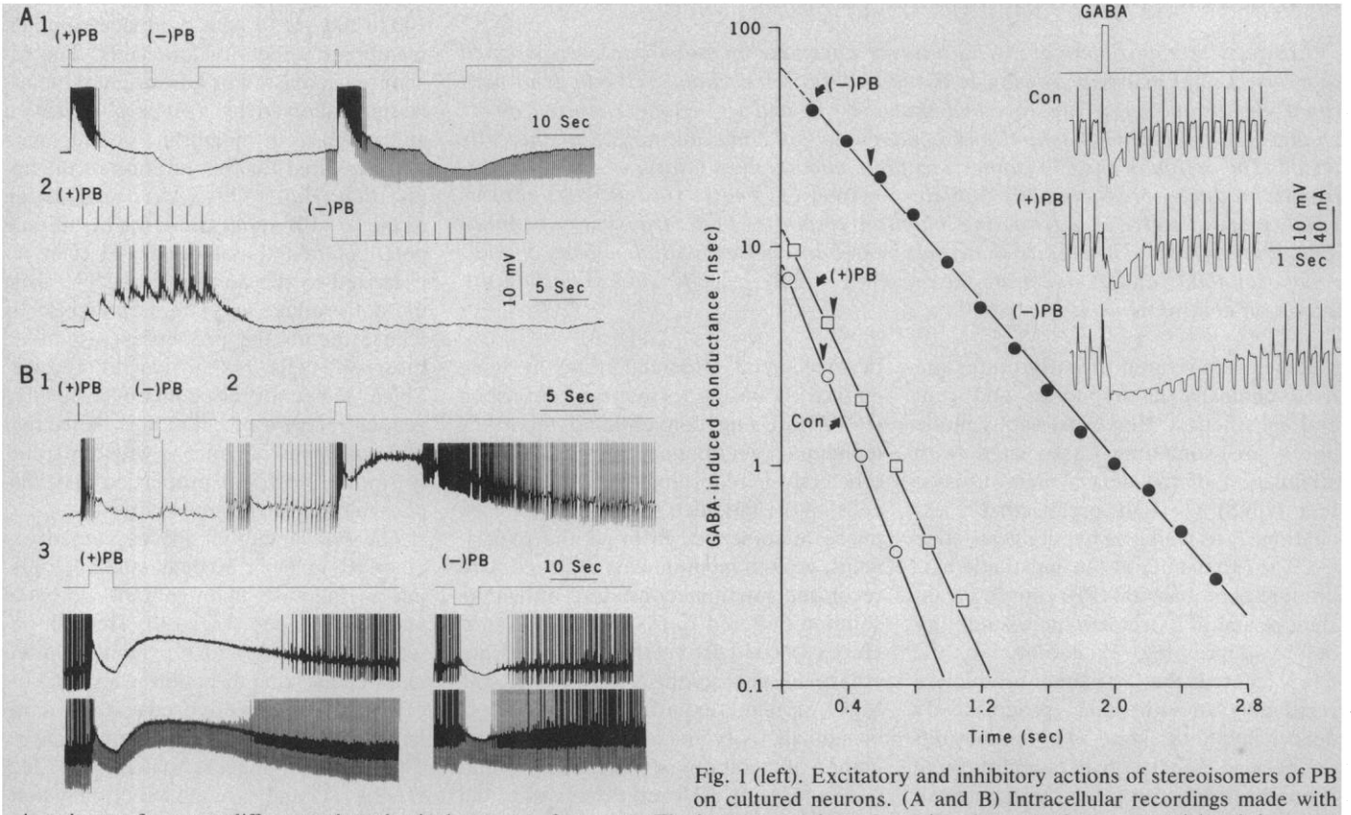

Fig. 1 (left). Excitatory and inhibitory actions of stereoisomers of PB on cultured neurons. (A and B) Intracellular recordings made with micropipettes from two different cultured spinal neurons of a mouse. The lower traces in each section show membrane potential and the upper traces indicate the duration of drug application delivered by pressure from pipettes positioned close to the recorded cells. The pipettes contained either (+)-PB or (−)-PB in 100 μM concentration. We added 10 mM $MgCl_2$ in both experiments to block ongoing synaptic activity. (A_1) Brief application of (+)-PB (indicated by upward deflection on upper trace) rapidly depolarizes the cell causing excitation that outlasts the application period. Application of (−)-PB (indicated by downward deflection on upper trace) hyperpolarizes the cell and increases membrane conductance, as reflected in the depression of voltage responses to constant-current (−0.25 nA) stimuli during the hyperpolarizing response. Resting membrane potential: −51 mV. (A_2) Membrane is polarized to − 61 mV. Left: Repeated brief applications of (+)-PB cause depolarizing responses that summate; (+)-PB also evokes synaptic activity as reflected in the appearance of discrete synaptic potentials superimposed on the depolarizing responses. Right: In the presence of sustained application of (−)-PB (monitored on the upper trace) the depolarization and evoked synaptic events caused by the same repeated brief applications of (+)-PB are blocked. The (−) isomer does not hyperpolarize in this case because the holding potential is close to the null potential for the (−)-PB response. (B_1) Dual effects of the isomers. Application of 100 μM (+)-PB for 50 msec is strongly excitatory; application of 100 μM (−)-PB for 500 msec is weakly excitatory. Membrane potential: − 50 mV. (B_2) One-second applications of the isomers to the same cell show that (−)-PB transiently excites and then inhibits spontaneous firing, whereas (+)-PB causes prolonged depolarization that first excites and then depolarizes to a potential which inactivates spike generation. (B_3) When the isomers were applied for 5 seconds (+)-PB induced a complex response consisting of an initial depolarization followed by a progressive hyperpolarization. After termination of drug application, the membrane potential first depolarizes and then repolarizes to control levels. A 5-second application of (−)-PB leads to a sustained inhibition of action potential activity through an increase in membrane potential and conductance as reflected in the decrease in voltage responses (the downward deflections) to constant current (−0.2 nA) stimuli. Membrane potential in B_2 and B_3: − 46 mV. Fig. 2 (right). Potentiation of GABA-induced membrane conductance by PB isomers. Membrane potential traces of responses to iontophoretic application of GABA (marked on top trace) before (*Con*) and during pressure application of either 100 μM (+)-PB or 100 μM (−)-PB. Upward-going events are "off" responses. Downward-going deflections are voltage responses to constant current stimuli (−0.25 nA). Plot of GABA-induced conductance under the three conditions shows that (+)-PB, slightly, and (−)-PB, markedly, enhance GABA-induced conductance and prolong the time constant of decay of the conductance response (marked by downward arrowheads). Resting potential: − 47 mV.

both sites are present, as when (−)-PB blocks excitatory responses to (+)-PB (Fig. 1, A_2).

The variable presence of the inhibitory responses on these cultured neurons contrasts with the ubiquitously present GABA responses (*11*), indicating that the inhibitory effects of PB are probably not mediated through activation of GABA receptors, as had been previously proposed (*8, 9*). The excitatory actions of (+)-PB and the inhibitory and GABA-potentiating actions of (−)-PB correlate well with the stimulant effects of (+)-PB and the depressant effects of (−)-PB observed in vivo, suggesting that these cellular actions may be important in the clinical responses to the drugs (*13*). The electrophysiological data also provide evidence for the presence of several functionally and stereospecifically distinct sites of barbiturate action and indicate that steric, in addition to hydrophobic considerations (*14*), are important in determining the pharmacologic actions of pentobarbital.

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Synthesis of Lipid During Photosynthesis by Phytoplankton of the Southern Ocean

Abstract. *Assimilation of carbon-14 labeled bicarbonate into photosynthetic products was measured at four stations in the Southern Ocean. Phytoplankton populations incorporated as much as 80 percent of the fixed carbon into lipid under conditions of low temperatures (−0.2° to −1.8°C) and low light intensities. At higher temperatures (+0.3° to +0.8°C) and higher light intensities, incorporation into lipid accounted for less than 20 percent of the fixed carbon, synthesis of polysaccharide and protein being more prominent.*

Studies of primary productivity of the waters surrounding the Antarctic continent have emphasized mainly (i) the geographical distribution of productivity (*1*) and (ii) the question of temperature adaptation among the phytoplankton (*2*). To expand such studies of primary productivity in the Southern Ocean we have examined the synthesis of the major types of end products of phytoplankton photosynthesis—polysaccharide, lipid, and protein. The origin of this approach has been described (*3, 4*). In essence, this previous work—both with cultures of several marine algae and with natural phytoplankton from temperate, subtropical, and tropical waters—has suggested that environmental "stress" (limited nutrient availability or suboptimal light intensity) causes the cells to con-

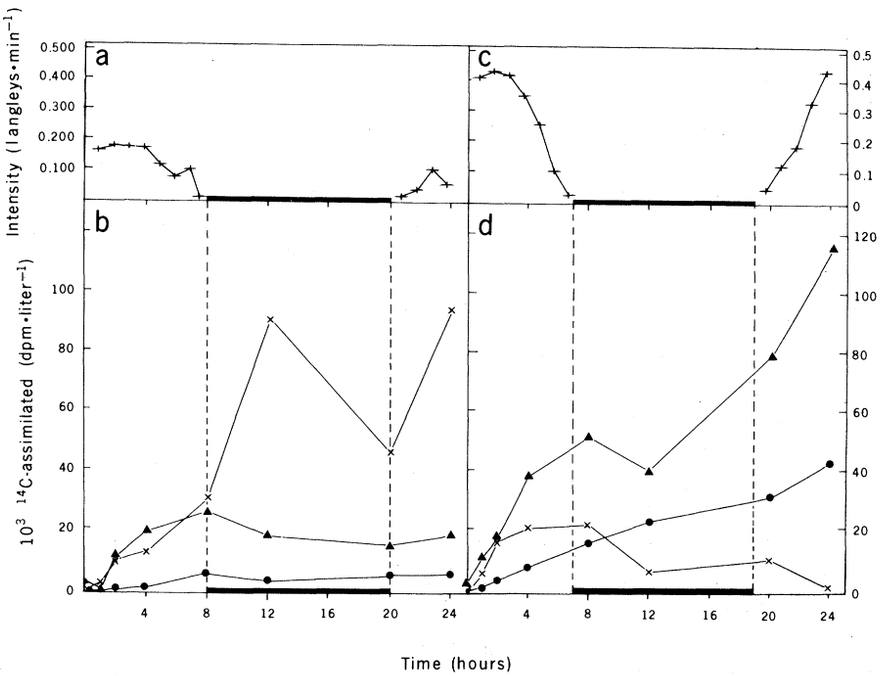

Fig. 1. Diurnal changes in the pattern of photosynthesis when 4 liters of surface water were incubated with ^{14}C-labeled bicarbonate at the ambient temperature and natural light. Data show the incorporation into lipid (crosses), protein (filled circles), and polysaccharide/metabolite pool material (filled triangles). (a) Pattern of photosynthesis observed at station 18 where the ambient temperature was − 1.0°C. (b) Pattern of photosynthesis at station 29, with an ambient temperature of 0.2°C.

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