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Suppression of Repetitive Firing of Neurons by Diphenylbarbituric Acid

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ABSTRACT

A slow outward current has been demonstrated in *Aplysia* giant neurons which serves to suppress repetitive firing. The barbiturates phenobarbital and pentobarbital enhance the slow outward current and the suppression of repetitive firing. In this study, the effects of diphenylbarbituric acid, which shows anticonvulsant activity in mice and rats but possesses minimal sedative properties, were tested on slow outward current and firing behavior. Diphenylbarbituric acid enhances slow outward current and suppresses repetitive firing at lower concentrations than phenobarbital and pentobarbital. Because diphenylbarbituric acid is effective at enhancing slow outward current but does not produce sedation, this property of barbiturates is apparently not associated with the sedative properties of these drugs, but rather is important for the anticonvulsant effects.

A common problem of many anticonvulsants is the incidence of unpleasant side effects, especially drowsiness (Rall and Schleifer, 1980). This might be explained by the fact that some anticonvulsants are neurodepressants that are not totally selective in suppressing the excess neuronal activity associated with seizures. An ideal anticonvulsant would effectively suppress "excessive" neuronal activity while minimally affecting "normal" brain function.

DPB (McElvain, 1935) is a compound structurally similar to two of the most commonly used anticonvulsants, phenobarbital and diphenylhydantoin (see fig. 1), and has been shown to prevent pentylenetetrazol- and maximal electroshock-induced seizures and death in mice (Raines *et al.*, 1973). Although DPB was effective in these anticonvulsant drug screens, there was little evidence of neurotoxicity at any dose. The results of this study indicated that DPB may be a very selective anticonvulsant agent.

Work in this laboratory has demonstrated that one neuronal action of the barbiturates is the enhancement of a voltage sensitive slow outward current. This current normally serves to cause spike frequency adaptation in response to a prolonged stimulation (Cote *et al.*, 1978). Pharmacological enhancement of this current by barbiturates results in an increased rate of adaptation and thus an inhibition of prolonged neuronal firing (Zbicz and Wilson, 1981).

In this study we show that DPB, a relatively selective *anticonvulsant* barbiturate, enhances slow outward current and adaptation, which suggests that the enhancement of spike frequency adaptation by barbiturates may be one mechanism by which they achieve anticonvulsant effects.

Methods

Aplysia californica (Pacific Biomarine, Venice, CA) were kept in a marine aquarium at 15°C for a period of less than 2 weeks before use. The abdominal and circumesophageal ring ganglia were excised through a midline incision of the foot.

Ganglia were pinned out in a Sylgard (Dow Chemical, Midland, MI) lined chamber and were perfused continuously with an artificial sea water solution. This solution consisted of (millimolar): NaCl, 575; KCl, 10; $NaHCO_3$, 2; $MgSO_4$, 30; and $MgCl_2$, 20 and was adjusted to pH 7.8 before each experiment. In most experiments an additional 50 mM $MgCl_2$ was added to the artificial sea water to bring the total Mg^{++} concentration to 100 mM. This high Mg^{++} solution was used to suppress synaptic activity and simplify the measurements of slow membrane currents. The temperature within the perfusion chamber was kept at 20 ± 0.5°C by means of a feedback controlled Peltier cooling device.

In each of the experiments one of the giant cells (LP1, Hughes and Tauc, 1961 or R2, Frazier *et al.*, 1967) was impaled through the connective tissue sheath with a 0.6 to 1.2 megohm microelectrode filled with 1.5 M KCl. The cells were then voltage clamped using a switching single electrode voltage clamp (Wilson and Goldner, 1975).

Prolonged depolarizations in voltage clamp and interstimulus interval were kept constant during the course of an experiment by a digital timing controller. Currents elicited during prolonged depolarizing voltage clamp pulses were quantitated by measuring the tail currents after the return to holding potential. It has been shown that slow outward current tails can be represented by the sum of exponential decay processes (components) (Evans *et al.*, 1981). The tail currents were quantitated by an exponential "peeling" process analogous to that used in compartmental analysis (Zierler, 1981). A BASIC program was used

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ABBREVIATIONS: DPB, diphenylbarbituric acid; GABA, γ-aminobutyric acid.

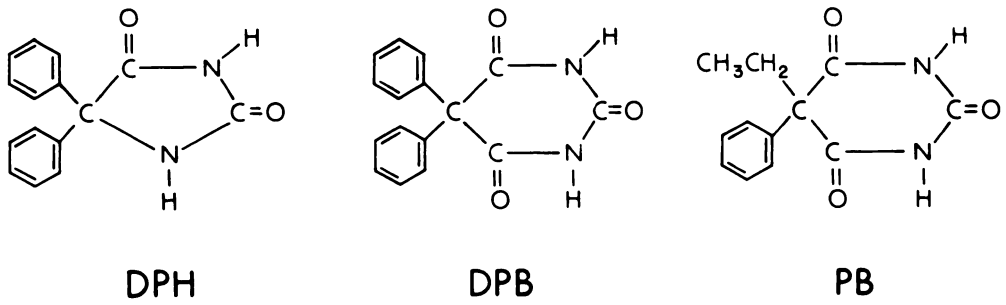

Fig. 1. Structures of diphenylhydantoin (DPH), DPB and phenobarbital (PB). Notice the similarities in the ring structures and the substituents of the three compounds.

which determined: 1) the number of decay processes, 2) the rate constants and 3) the zero time intercepts. In all cases where an equally good fit could be obtained by models with different numbers of compartments, the simplest model (that with the fewest components) was used. This method of analysis will be discussed further in a forthcoming communication. DPB was obtained compliments of Dr. A. Raines, Georgetown University.

Results

Effects of DPB on adaptation. Adaptation in *Aplysia* giant neurons occurs in two phases. In response to a constant current depolarizing stimulus there is an initial rapid decrement in the firing rate which occurs within the first 5 sec followed by a much slower rate of adaptation which occurs over a period of 10s of sec. DPB enhances the late phase of adaptation in a concentration-dependent manner. Increasing effects are seen in the range of 2 to 80 μM. This is at least an order of magnitude lower than the minimum effective concentration for phenobarbital (50 μM, Zbicz and Wilson, 1981).

In some experiments there was a brief (2–5 sec) pause in the firing which occurred early in the stimulation (within the first 10 sec). When this was the case, DPB caused a concentration-related prolongation of the pause. The results in figure 2, which are from a giant neuron that exhibited such a pause, depict the effect of increasing doses of DPB on spike frequency adaptation. Although there is no effect on the initial firing rate, the increase in interspike interval during the final portion of the constant current stimulus is concentration-dependent. The increase in late adaptation is reversible and washes slowly over a period of hours. The enhancement of late adaptation by DPB occurred in all giant neurons studied ($n = 13$).

Effects of DPB on slow outward current. During a prolonged voltage clamp depolarization to near spike threshold, a series of slow conductance changes occurs (Cote *et al.*, 1978; Zbicz, 1979). During the first few seconds of depolarization there is an initial inward current followed by a biphasic increase in outward current (see fig. 3). The two phases of outward current are 1) an initial rapid phase that is complete within 5 to 10 sec and 2) a later phase with a much slower time course. The slow phase of outward current is enhanced by anticonvulsant concentrations of phenobarbital (Zbicz and Wilson, 1981).

In this series of experiments we used voltage clamp steps to near spike threshold to elicit slow outward current. The cells were held at −50 mV and then step depolarized to −30 mV for 60 sec. Upon returning to −50 mV, tail currents were evident that represented the decay of the slow outward current that had been activated during the depolarization. DPB caused a concentration-related increase in the rate of rise of slow outward current as well as in the amplitude of the tail current (fig. 3). We have used the tail currents as a means to quantitate the slow outward current elicited during the depolarizing step. The tail currents are potassium sensitive and can be represented by the sum of two exponential decay processes. These two processes have half-lives of approximately 10 and 70 sec at −50 mV and 20°C (Evans *et al.*, 1981).

The amplitude (zero time intercept) of the slow component of tail current was increased by DPB in a concentrated-related manner in 12 of 12 giant neurons. Figure 4 shows semilogarithmic plots of the tail currents from a typical experiment. DPB causes a parallel upward shift in the late tail current, indicating an increase in the initial amplitude but not the kinetics of slow tail current decay. Significant increases in the amplitude of the slow component were seen with concentrations above 2 μM DPB, whereas above 20 μM only slight increases (and sometimes decreases) were seen. Figure 5 shows the concentration-response relationships obtained with DPB for the effects on the amplitude of the two components of tail decay (A1 and A2). Although DPB caused a dose-related increase in the amplitude of the tail current, the rate constants of the two components were not significantly affected by concentrations up to 20 μM.

Discussion

In this study, the effects of the specifically anticonvulsant-barbiturate, DPB, have been tested on the slow outward current of *Aplysia* giant neurons. In order to quantitate the effects of this compound on slow outward current, a kinetic analysis of the decay phase (tail current) was utilized. This method provided a reliable, reproducible means of characterizing alterations in the slow outward current.

Using this method, it has been demonstrated that DPB causes a relatively selective increase in one component of tail current decay, *i.e.*, in the initial amplitude of the slower phase. This effect was concentration-related and approached a maximum. Neither the rate of decay of the slow phase nor any parameters of the fast phase were sensitive to DPB in moderate concentrations (less than 20 μM). Thus, the amplitude of the slow phase was used as an assay of slow outward current enhancement in order to determine the concentration-response relationship for this compound.

According to this assay, the EC_{50} of DPB is approximately 3.5 μM, with effects being seen with as little as 1 μM in some cases. This compares favorably with the effects of DPB on slow adaptation in these neurons. Here also effects were seen with concentrations greater than 1 μM. This contrasts strongly with the effects of phenobarbital, which causes appreciable effects on adaptation and slow outward current only with concentrations of at least 50 μM (Zbicz and Wilson, 1981). Thus, DPB has similar actions to phenobarbital in this test system, but its

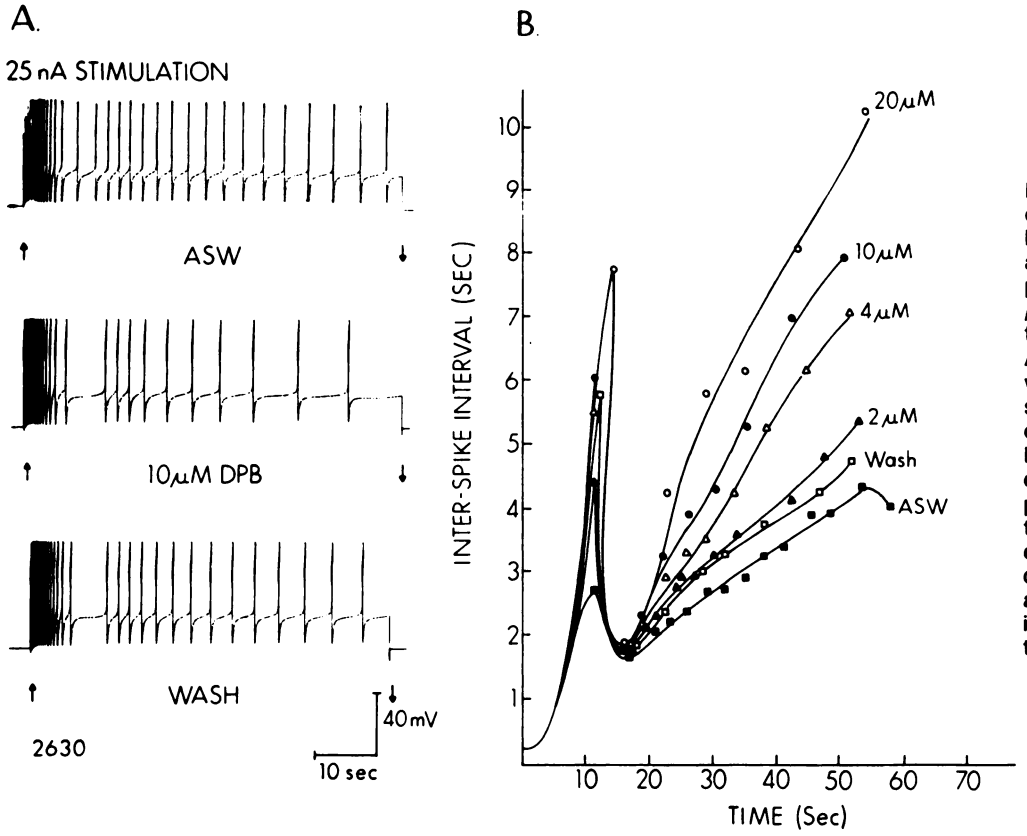

Fig. 2. Typical repetitive firing pattern of giant cell R2 and changes produced by DPB. A, adaptation in response to a 60 sec, 25 nA constant current depolarization obtained in control, with 10 μM DPB and with wash. Arrows mark the beginning and end of the stimulus. Action potential amplitude in the figure was attenuated by the frequency response of the chart recorder. B, to elucidate the relationship between DPB concentration and adaptation, data from the experiment in part A are plotted as the interspike interval *vs.* time of depolarization. DPB causes a concentration-related reversible increase in adaptation as evidenced by an increased rate of development of interspke interval. Adaptation within the first 5 sec was unchanged by DPB.

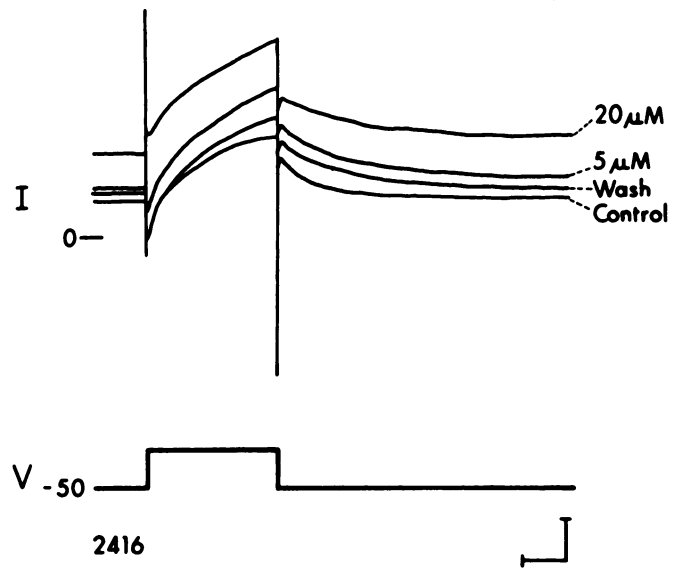

Fig. 3. Slow outward current produced by a 60 sec depolarization to −30 mV from a holding potential of −50 mV. DPB produces a reversible, concentration-dependent increase in the rate of rise of slow outward as well as the tail current amplitude. Upper trace is current (I, upward is outward) and lower trace is voltage (V). Calibration, horizontal = 20 sec; vertical = 20 mV, 10nA.

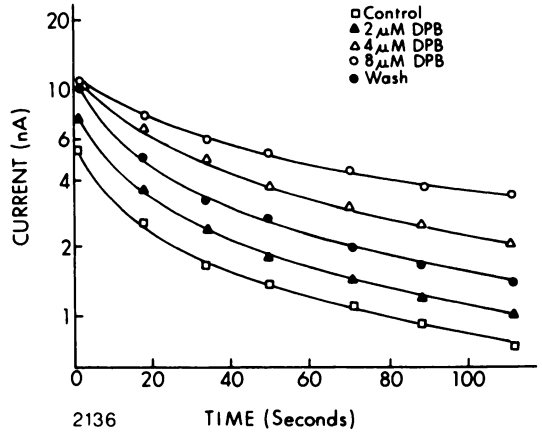

Fig. 4. Semi-log plots of tail currents obtained in control and with DPB. The addition of DPB results in a concentration-related, reversible increase in the amplitude of tail currents without affecting the kinetics.

potency is approximately one order of magnitude lower than phenobarbital.

This is a significant finding because DPB is a very selective anticonvulsant barbiturate with little neurotoxicity (sedation) in mice and rats (Raines *et al.*, 1975, 1979). The fact that DPB is very potent at enhancing slow outward current and spike frequency adaptation in *Aplysia* neurons, in conjunction with the fact that DPB possesses little sedative activity, indicates that enhancement of spike frequency adaptation (*via* increased slow outward current) by barbiturates may not be related to the neurotoxic effects but rather may be associated with the anticonvulsant activity of this group of compounds.

A question that is raised by this study is the following: if enhancement of slow outward current by barbiturates is a mechanism by which a selective anticonvulsant effect may be realized, then what actions of the barbiturates underlie the sedative properties of this group of compounds? One possibility is through the enhancement of GABAergic inhibition. Fanelli *et al.* (1981) demonstrated that in the rat hippocampus the sedative barbiturates secobarbital and pentobarbital enhanced GABA-dependent [^{3}H]flunitrazepam binding and enhanced the

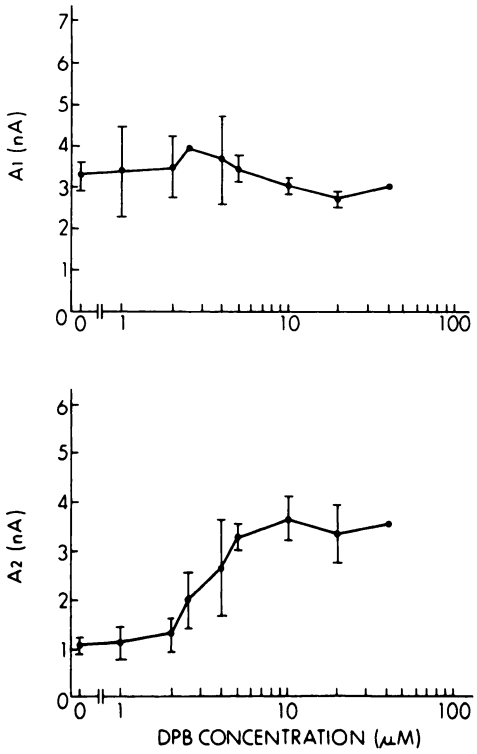

Fig. 5. Concentration-response relationship obtained with DPB for the increase in amplitude of tail components. A1 and A2 represent the amplitude (zero time intercept) of the fast and slow component of tail current, respectively. Averaged results of 11 experiments. Error bars represent ± S.E.M.

process of GABA-dependent recurrent inhibition. On the other hand, the selectively anticonvulsant barbiturates, phenobarbital and DPB, were ineffective at enhancing either of these responses. A study of Whittle and Turner (1982) indicated that sedative barbiturates are more effective than anticonvulsant barbiturates at enhancing GABA binding in membranes from rat brain cortex. MacDonald and Barker (1978) showed that the sedative barbiturate pentobarbital was more effective than the anticonvulsant barbiturate phenobarbital at enhancing and mimicking GABA responses in cultured spinal cord neurons.

One might then postulate that anticonvulsant barbiturates are those that enhance spike frequency adaptation *via* slow outward current, whereas barbiturates that enhance GABA responses would possess sedative activity. The relative effectiveness of specific barbiturates in enhancing these two effects would then determine their therapeutic usefulness as anticonvulsants. The findings of these works may therefore prove useful in the development of anticonvulsant drugs.

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