needed for confirmation of this possibility. We think it unlikely that the somatostatin in the upper gastrointestinal tract and pancreas is of hypothalamic origin because of the large quantities found, but this possibility has not been excluded.

AKIRA ARIMURA
HARUKO SATO
ANDRE DUPONT
NOZOMU NISHI
ANDREW V. SCHALLY

*Department of Medicine, Tulane University School of Medicine,* and *Endocrine and Polypeptide Laboratories, Veterans Administration Hospital, New Orleans, Louisiana 70112*

### References and Notes

1. Amino acid abbreviations are as follows: Ala, alanine; Gly, glycine; Cys, cysteine, Lys, lysine; Asn, asparagine; Phe, phenylalanine; Trp, tryptophan, Thr, threonine; Ser, serine; Glu, glutamic acid; Arg, arginine; Tyr, tyrosine; Pro, proline; Leu, leucine; Val, valine; and His, histidine.
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## Diphenylhydantoin: Action of a Common Anticonvulsant on Bursting Pacemaker Cells in *Aplysia*

Abstract. *A commonly used anticonvulsant, diphenylhydantoin (Dilantin), decreases the bursting pacemaker activity in certain cells of* Aplysia. *Dilantin decreases this bursting activity whether it is endogenous to the cell or induced by a convulsant agent. The sodium-dependent negative resistance characteristic which is essential for bursting behavior is reduced in the presence of Dilantin.*

Although the anticonvulsant action of diphenylhydantoin (Dilantin) has been studied extensively for several years (*1*), the mechanisms involved remain somewhat obscure. Several theories have been proposed to explain the action of Dilantin. One hypothesis has been that Dilantin increases the adenosine triphosphatase–dependent Na-K active transport (*1, 2*). This theory, however, has been brought into serious question by recent data on the crayfish stretch receptor and other preparations (*3, 4*). Other studies, instead, have indicated that Dilantin decreases the downhill flow of sodium ions during the action potential (*5*) and the sodium-dependent excitatory postsynaptic potentials (EPSP's) by a postsynaptic mechanism (*6*).

Recently, considerable information has become available on the mechanisms underlying bursting properties in the bursting pacemaker cells of *Aplysia* (*7, 8*). In a normal bursting pacemaker cell, a region of negative slope resistance (*8*) exists in the steady-state current-voltage (*I-V*) curve in the range of the potential oscillations. The negative resistance characteristic (NRC) has been shown to be due to a regenerative inward sodium current (*9*). The hyperpolarizing phase of the oscillation is produced by an outward potassium current which is activated during the depolarizing

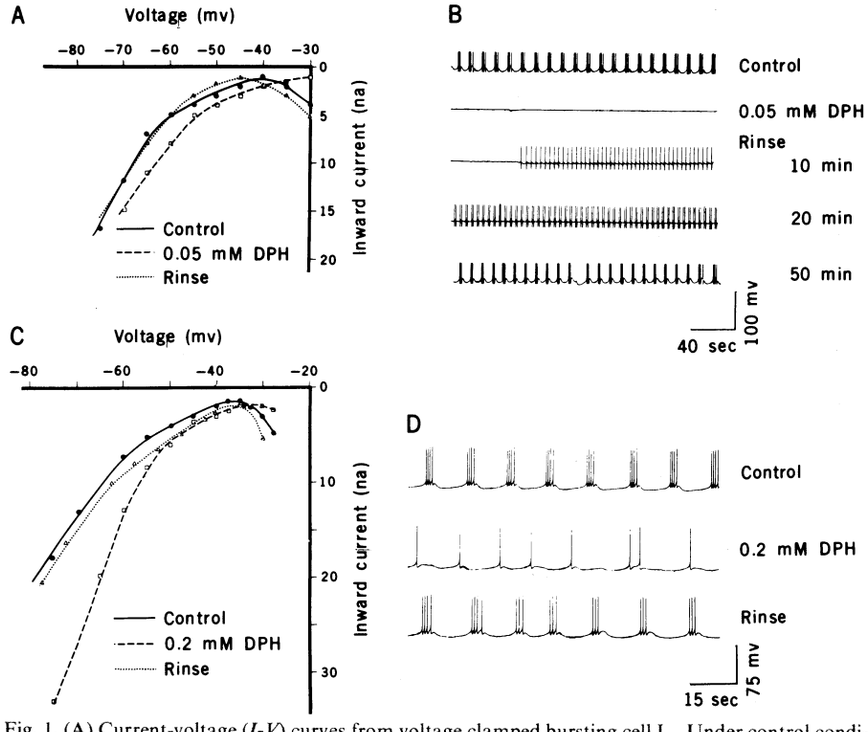


Fig. 1. (A) Current-voltage (*I-V*) curves from voltage clamped bursting cell $L_6$. Under control conditions there is a region of negative slope resistance in the *I-V* curve in the range of the potential oscillation. After 30 minutes of perfusion with 0.05 m*M* Dilantin this NRC has disappeared along with the bursting activity. The region of negative resistance returns with rinse. (B) Effects of Dilantin on bursting pacemaker cell. Cell $L_6$ is bursting regularly during control. After 30 minutes of perfusion with 0.05 m*M* Dilantin bursting activity has disappeared. The cell slowly returns to control condition after 10, 20, and 50 minutes of rinse with normal seawater. (The true amplitude of the action potentials cannot be taken from this figure because the spikes are being clipped by the pen recorder.) (C) Current-voltage curves from voltage clamped bursting cell $L_6$. The bursting decreases but does not fully disappear after more than 60 minutes of perfusion with 0.2 m*M* Dilantin. The NRC in the *I-V* curve decreases but is still present with Dilantin. (D) Dilantin (0.2 m*M*) is applied to cell $L_6$ from another ganglion. The bursting pattern is nearly abolished after 60 minutes of perfusion. The cell, however, is still firing spontaneously but at irregular intervals. Rinsing returns the cell to the control level of activity.

phase of the oscillation (*9*). Several reports indicate that normally silent cells can acquire bursting characteristics (*10*), with a region of negative slope resistance appearing in the steady-state *I-V* curve (*11*), when exposed to convulsant agents such as pentylenetetrazol (PTZ) and strychnine. Since Dilantin has been shown to decrease the inward sodium current of the action potential in squid axon (*5*) and the sodium-dependent EPSP's in *Aplysia* (*6*), the sodium-dependent NRC was of particular interest to our investigation. We focused on the effects of Dilantin on cells which normally exhibit this bursting pattern and on silent cells which had been induced to bursting by the application of a convulsant agent.

The abdominal ganglion of *Aplysia californica* was removed from the animal and two KCl electrodes were used to impale one of the bursting cells ($L_2$ to $L_6$) or the silent giant cell $R_2$ (*7*). Voltage measurements were made with conventional techniques, displayed on an oscilloscope and chart recorder, and stored on magnetic tape. Voltage clamping was employed when necessary using standard techniques (*7, 8*), except the axonal charging currents prevented us from observing events sooner than 100 msec after a voltage step command (*12*). Voltage clamping is required to investigate the mechanisms underlying bursting activity, since it is impossible to hold a steady membrane potential within the range of the oscillation merely by passing current (*8*). Changes in the bursting pattern were studied both by observing directly and by noting alterations in the steady-state *I-V* characteristics of the voltage clamped cell. The *I-V* curves were obtained with a series of hyperpolarizing voltage step commands from a holding potential at the peak of the depolarizing phase of the oscillation. The ganglion was continuously perfused with artificial seawater (ASW) and ASW containing Dilantin or PTZ, or both. Temperature was controlled at 20°C.

Figure 1, A and B, shows the results of perfusing 0.05 m*M* Dilantin (*p*H 8.0) on bursting cell $L_6$. After 30 minutes, bursting activity had ceased. If a depolarizing transmembrane current was applied, a steady firing pattern resulted (not shown). The cell was voltage clamped and the *I-V* curve was obtained by a series of hyperpolarizing voltage step commands before and during the perfusion. The NRC that was present during control had disappeared with Dilantin. Rinsing with normal ASW reinstated both the bursting pattern and the NRC. In some cells the bursting pattern was not completely suppressed even after more than 60 minutes of perfusion with Dilantin. A weak bursting pattern remained with usually no more than one impulse per burst (Fig. 1D). In these cells, during perfusion with Dilantin, the NRC had decreased, but was not abolished (Fig. 1C). The concentration of Dilantin ranged between 0.02 and 0.2 m*M*, and similar results were observed in all experiments. A concentration of 0.05 m*M* is equivalent to 13.6 $\mu$g/ml and is in the therapeutic range for total serum diphenylhydantoin in humans. In most of the cells studied a decrease in resistance [increase in anomalous rectification (*13*)] was observed for hyperpolarizing voltage step commands during Dilantin perfusion, especially at higher concentrations (0.1 to 0.2 m*M*) (see Fig. 1C). This datum agrees with the decrease in resistance measured by Ayala and Lin (*3*) with Dilantin on the crayfish stretch receptor.

We then applied 20 m*M* PTZ to the ganglion while recording from the silent giant cell $R_2$. As reported by others (*10, 11*) slow membrane potential oscillations gradually developed and became spontaneous bursts. A region of negative resistance appeared in the *I-V* curve (Fig. 2). Adding 0.1 m*M* Dilantin to the perfusion bath containing PTZ reversed the action of this convulsant agent by decreasing the bursting activity and the NRC. Returning to PTZ alone brought back the bursting pattern and negative resistance. These effects were reversed after the ganglion was rinsed with normal ASW.

Our observation that Dilantin decreases the bursting pacemaker activity in *Aplysia* is consistent with its proposed action on the downhill flux of sodium ions, since the regenerative inward sodium current (negative resistance) is abolished with Dilantin. This action of Dilantin takes place whether the bursting activity is endogenous to the cell or induced by a convulsant agent. Recently, the external calcium ion concentration has been implicated as a mechanism in the regulation of the bursting pacemaker response in *Aplysia* and the land snail (*14*). It is possible that the effects of Dilantin on bursting cells reported here could involve the activity of calcium, but this idea is presently unsupported by data.

The results of these experiments on *Aplysia* and the data of others with convulsant agents (*10, 11*) may be applicable to the generation of the paroxysmal depolarizing shift (PDS) seen in cortical spike foci. One theory pertaining to the generation of the PDS (*15*) is concerned primarily with changes in synaptic efficacy; however, neuronal bursting activity caused by a region of negative resistance may also be involved in the development of the PDS by affecting the membrane characteristics of either the pyramidal cell dis-

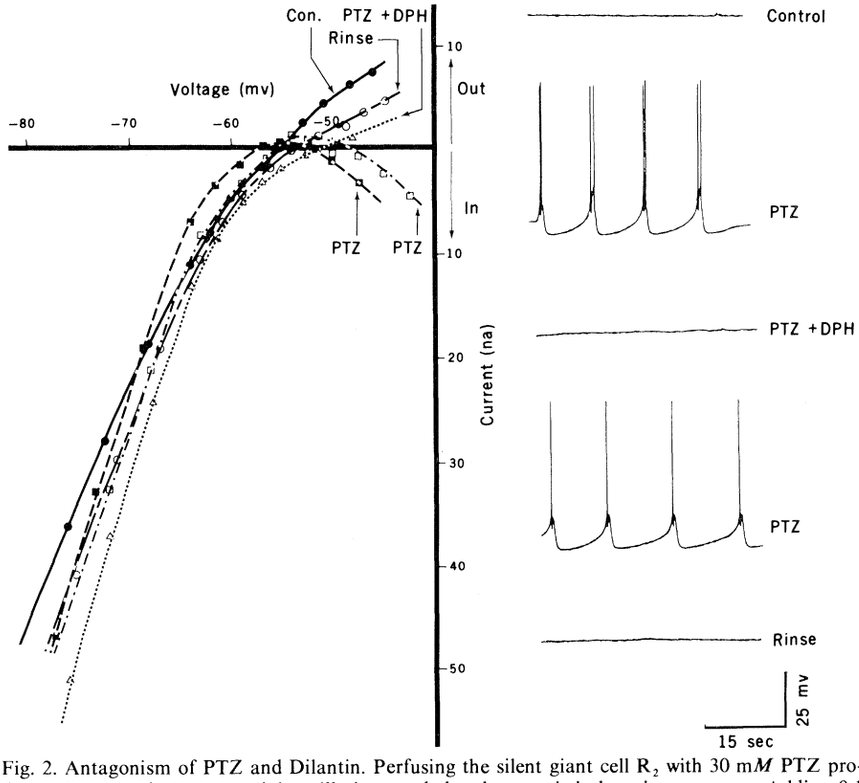


Fig. 2. Antagonism of PTZ and Dilantin. Perfusing the silent giant cell $R_2$ with 30 m*M* PTZ produces slow membrane potential oscillations and the characteristic bursting response. Adding 0.1 m*M* Dilantin to the seawater containing PTZ reverses the action of this convulsant agent, since the bursting activity disappears. Returning to PTZ alone reinstates the bursting, and rinsing with normal seawater returns the cell to its original silent state. Current-voltage curves show a region of negative resistance, which is present during PTZ perfusion but disappears when Dilantin is added.

playing the PDS or the interneurons of the recurrent pathways. This model based on bursting behavior is purely speculative at this time, since information concerning the latent ability of neocortical cells to acquire bursting properties is not available. However, the finding that the convulsant and anticonvulsant agents affect the NRC and bursting properties in this invertebrate model are highly suggestive that a similar mechanism may occur in mammalian neurons.

DANIEL JOHNSTON, GIOVANNI F. AYALA
*Department of Neurology* and
*Biomedical Engineering Program,*
*University of Minnesota,*
*Minneapolis 55455*

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## Loss of X-Cells in Lateral Geniculate Nucleus with Monocular Paralysis: Neural Plasticity in the Adult Cat

Abstract. *Chronic immobilization of one eye by cranial nerve resection in adult cats led to selective but substantial loss of X-cells in the binocular segment of the dorsal lateral geniculate nucleus.*

No plasticity is usually seen in the visual systems of adult animals subjected to aberrations of visual input (*1*, *2*). In contrast, the neural plasticity displayed by kittens reared under conditions of abnormal interocular interaction is well documented (*1*, *3*).

Recently, however, Buchtel *et al.* (*4*) have observed behavioral as well as physiological changes following chronic monocular paralysis in adult cats. In a more detailed analysis of the physiological effects of monocular paralysis, Fiorentini and Maffei (*5*) found a loss of binocularity in simple cells of visual cortex, while the binocularity of complex cells was apparently unaffected. After chronic paralysis, fewer than 15 percent of simple cells could be driven binocularly, whereas 95 percent were binocular during the first week of immobilization. Furthermore, this loss of binocularity in simple cells occurred rather abruptly about the seventh or eighth day of monocular paralysis and remained unaltered over several months regardless of any recovery of oculomotricity.

We have investigated the possibility that monocular paralysis in adult cats produces changes in the lateral geniculate nucleus (LGNd) in addition to the effects described in cortex. Certain specific changes in the LGNd, following chronic monocular paralysis, may be expected from the parallel processing model of connectivity within the geniculostriate system (*6*). This model states that visual information is conveyed from retina to LGNd and finally to visual cortex by two parallel pathways, one containing X-cells and the other Y-cells. The axons of X-type retinal ganglion cells contact X-cells in the LGNd which then contact primarily simple cells in the visual cortex. The Y-cells in the lateral geniculate receive input from Y-cells in the retina and contact complex cells directly. Since Fiorentini and Maffei found that monocular paralysis has its principal effect on simple cells, we anticipated that we would see a selective disruption of the X-cells in the LGNd. Therefore, we investigated the effects of chronic eye immobilization on the relative proportions of X- and Y-cells in the cat's lateral geniculate nucleus.

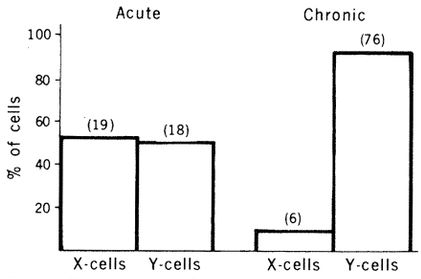


Fig. 1. Comparison of acute and chronic monocular paralysis with respect to the percentage of X- and Y-cells in the binocular segment of the LGNd. With monocular paralysis lasting 3 days or less (acute condition), X- and Y-units were encountered with roughly equal frequency. After more than 14 days of monocular paralysis (chronic condition) the percentage of X-units had declined to less than 10 percent, while that of Y-cells had risen to more than 90 percent. Numbers in parentheses refer to absolute number of units represented by these percentages.

Under sodium pentobarbital anesthesia, one eye in each of six cats was surgically immobilized by transection of cranial nerves III, IV, and VI at the common point of their entry into the orbit. During the same surgical procedure, the animal was prepared for semichronic microelectrode recording.

During recording sessions the animal was sedated, but not anesthetized, with a mixture of acepromazine maleate and sodium pentobarbital. The head was fixed in the stereotaxic plane by means of chronically implanted bolts attached to a metal mount (*7*). Complete muscular paralysis was unnecessary since cells under investigation were driven by the surgically immobilized eye. The eyes were protected by plano contact lenses, and refractive errors were corrected with spectacle lenses, so that sharp focus was obtained on a tangent screen 1 m from the cat's eye. The projections of the optic disc and the area centralis of each paralyzed eye were estimated on the tangent screen after the method of Fernald and Chase (*8*), and receptive fields were located and mapped with respect to these retinal landmarks. Each recording session lasted from 7 to 8 hours, and cats were allowed to recover completely before further recording.

Action potentials from units responsive to illumination of the immobilized eye were recorded with stainless steel microelectrodes, amplified on a WPI DAM 5 preamplifier and a Grass a-c amplifier, and stored on magnetic tape for subsequent analysis. Units were classified as X or Y on the basis of visual tests such as those described by Enroth-Cugell and Robson (*9*), Cleland, Dubin, and Levick (*10*), Fukada (*11*), Fukada and Saito (*12*), Cleland, Levick, and Sanderson (*13*), and Stone and Hoffmann (*14*). Ambiguous cases were decided by the radial grating method described by Dubin (*15*).