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Diphenylhydantoin Inhibition of Sodium Conductance in Squid Giant Axon

(dilantin/antiarrhythmic/voltage clamp/anticonvulsant/potassium conductance)

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ABSTRACT **Diphenylhydantoin, in concentrations of 5–50 μM, decreases the early, transient (sodium) currents of voltage-clamped squid giant axons. These effects are dose-dependent and largely reversible. It appears that diphenylhydantoin reduces the number of open, early, transient (sodium) channels.**

Among the many biological effects of diphenylhydantoin, its antiarrhythmic action (1, 2) has focused attention upon its effects on excitable membranes outside of brain. Although this drug has been widely used in patient care (3), and voluminously investigated (3, 4), no clear-cut ionic mechanism of action for it on any excitable membrane has emerged. In peripheral nerve this compound has, nonspecially, been considered to be a membrane "stabilizer" because it prevented or interrupted repetitive electrical activity induced by various means (5–7). However, a decrease in the maximum rate of rise of the action potential in isolated rat atrial fibers indirectly suggests that this drug can impair the sodium mechanism involved in the genesis of the action potential (8, 9). In this report, we describe ion-specific effects of diphenylhydantoin in the squid giant axon.

METHODS

In order to evaluate the effect of diphenylhydantoin on the transient and steady-state ionic currents in terms of the Hodgkin–Huxley relationships (10), we have studied the effect of the drug (made up from a commercially available powder of the sodium salt) on the voltage-dependent currents in voltage-clamped giant axons of the squid (the last stellar nerve of *Loligo pealei*). Both step (11) and ramp-clamp (12) techniques were used. Temperature was held between 3 and 8°, within ±1.0° during any given experiment; the external artificial sea water was potassium-free; 0.5 M KF solution buffered with Tris to pH 7.5 replaced the axoplasm; and chamber mixing, when external solutions were changed, was complete in about 1 min. The concentrations of diphenylhydantoin affecting squid axons in our experiments were in the same range as plasma concentrations that suppress ventricular arrhythmias in man (2).

RESULTS AND DISCUSSION

A voltage–current curve typical of the 13 axons studied is shown in Fig. 1. The lower curves show the relation of the maximal sodium current (the peak of the transient, inwardly directed current) to the membrane potentials to which the axon membrane was clamped. The upper curves show the same relation for the potassium current (the delayed, steady-state, positive current).

The principal result was a reduction of the transient, sodium currents. The effects with 10 μM diphenylhydantoin were greater than with 5 μM (Fig. 1), and 50 μM drug reduced the peak sodium current by about 75%. The effect was largely reversible, with "recovery" to 65–90% of the initial (control) sodium conductance upon return to control artificial sea water. In no experiment did the drug significantly change the voltage (i.e., less than 5–10 mV, representing the accuracy of the method used) at which the peak transient current occurred. Inconsistent, small (i.e., less than 0.1 msec) increases in the time to reach this peak current (for any given depolarizing step) lead us to tentatively conclude that no significant change in the time-to-peak sodium current occurred.

Diphenylhydantoin had little or no effect on the resting membrane potential or on the leakage currents. Its effects are probably not on the "resting elements" of the membrane, but rather upon the ionic channels that are associated with a change from the resting to the active state of the membrane. This permeability change (activated sodium conductance) is best characterized by the empirical formulations of Hodgkin and Huxley (10) as a product of three parameters (i.e., $g_{Na} = \bar{g}_{Na} m^3 h$). The absence of any voltage shift or delay in the time-to-peak sodium currents speak against the drug affecting the process of sodium activation (m), or sodium inactivation (h), or either of their time constants. Occasionally, small changes in the sodium reversal potential (as in Fig. 1) occurred (i.e., the potential where the sodium current is zero was shifted to the left after drug administration). Even when present, this effect was of insufficient magnitude to account for the observed decrease in the sodium current.

Thus, it appears that diphenylhydantoin principally alters the sodium conductance, $\bar{g}_{Na}$, presumably by directly blocking the activated channels through which the sodium ions normally enter the axon. This effect (decreasing $\bar{g}_{Na}$, without appreciably altering m or h) is similar to the effects of saxitoxin (13) and tetrodotoxin (14, 17), and is somewhat different from the effects of procaine (15).

Our use of fast (68 mV/msec) ramps permitted direct recording of sodium current–voltage curves without chemical or computational separation of the ionic currents (12). The time course of the diphenylhydantoin effect on sodium currents is best seen by a typical ramp voltage-clamp result (Fig. 2). The upper section of Fig. 2 shows the peak sodium

current decreasing exponentially with time after exposure to the drug. Calculation, by use of a least-squares computer program based on the method of successive aproximations (16), shows the effect has a half-time of 10 min. In confirmation of the step-clamp results, the time-to-peak sodium current (bottom section of Fig. 2) did not change during exposure to the drug (i.e., there was no change in the voltage at which the peak sodium current occurred).

In each of seven axons, the potassium currents were slightly smaller during exposure to diphenylhydantoin than during the previous control, but in no experiments did return to control solutions reverse this change. The range of reduction of the outwardly directed potassium currents (5–15%) was considerably less than that for the sodium currents (25–75%). 50 μM Diphenylhydantoin (which reduced the transient currents by 75%) had no greater effect on the steady-state currents than did 10 μM. The four successive procedures of Fig. 1 occupied about 2 hr, during which the curves shifted slightly and steadily to the right. It is not clear that this shift was influenced by diphenylhydantoin. Furthermore, there was no change in the potassium slope conductance. Each of these factors, coupled with the fact that reduced steady-state currents are a common sign of axon deterioration (15) occurring with repeated voltage-clamping, or with time alone, make the effects of the drug on potassium currents ambiguous. Diphenylhydantoin differs from procaine in this respect, since a 75% reduction of the sodium current induced by procaine in squid axon is accompanied by a clear and reversible 20% reduction in potassium current (15). Although the specificity of diphenylhydantoin for only the early, transient (sodium) channels is not unequivocally demonstrated by our results, it appears that this drug blocks the sodium channels more specifically than does procaine.

Diphenylhydantoin may be superficially compared with local anesthetics (e.g., procaine) since they both block conducted action potentials, primarily by diminishing the sodium conductance (15). However, unlike diphenylhydantoin procaine requires a 100-fold greater concentration (i.e., millimolar rather than micromolar) to produce the same degree of induced inhibition of sodium current, procaine changes the potassium current, and procaine changes the time constant of the Hodgkin–Huxley parameter m (15). A closer comparison might be drawn between diphenylhydantoin and saxitoxin (13) and tetrodotoxin (14, 17). The toxins and diphenylhydantoin alter $\bar{g}_{Na}$ without modifying the relevant time constants, and it appears that both types of drug do not significantly affect the potassium currents. The effect of diphenylhydantoin on $\bar{g}_{Na}$ accounts for its ability to block conducted action potentials (9), offers a mechanism for its decrease in the maximum rate of rise of the action potential seen in isolated myocardium (6), accounts for its stabilizing effects in peripheral nerve (7–9), and is the first clear description of its effects on ion-specific electrophysiological mechanisms. It is attractive to speculate that the effect of diphenylhydantoin on $\bar{g}_{Na}$ is related to its antiarrhythmic and anticonvulsant properties.

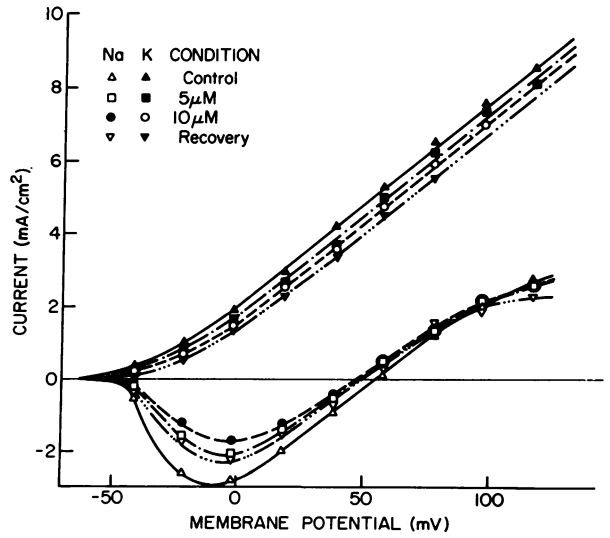

FIG. 1. Membrane current–voltage relationships, obtained from step voltage-clamp data, for the peak, sodium currents (*lower curves*), and for the delayed, potassium currents (*upper curves*). Concentration of diphenylhydantoin as shown.

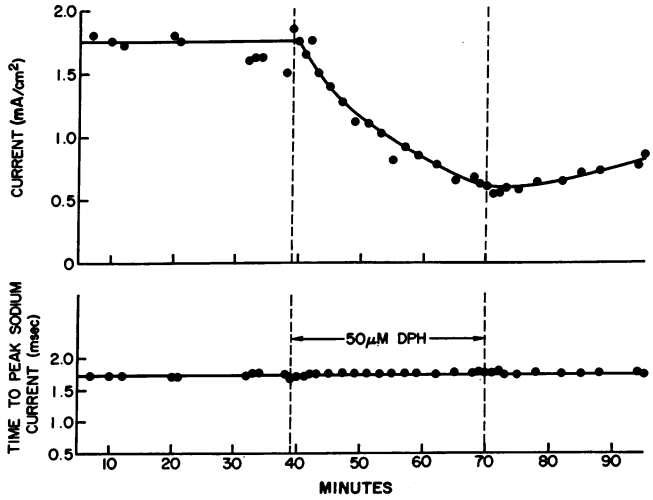

FIG. 2. The time-course of the effect of diphenylhydantoin (DPH) on the peak sodium current obtained from ramp voltage-clamp data.

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