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SLOW SODIUM CHANNEL INACTIVATION AND THE MODULATED RECEPTOR HYPOTHESIS

APPLICATION TO PHENOBARBITAL

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Summary

Consideration of the modulated receptor hypothesis leads to an interesting interpretation regarding sodium channel block (frog skeletal muscle) by phenobarbital. The inactive channel state appears to present a much higher affinity binding site to phenobarbital. In addition, use of hyperpolarizing prepulses underestimates the blocking potency for this kinetically fast drug.

Recent studies on the interactions of local anesthetics with axonal sodium channels have revealed an interesting channel state-dependent mode of action [1]. Sodium channel states associated with membrane depolarization are more easily blocked by local anesthetics. Thus, the sodium channel may be interpreted as a modulated receptor for these drugs. Several anticonvulsants have now been shown to produce frequency-dependent blocking actions [2] which suggests that certain features of the 'modulated receptor hypothesis' may hold for drugs within this class as well. Voltage clamp measurements in frog muscle fibers are presented here which support this contention. Phenobarbital preferentially blocks inactive sodium channels while unblocking may primarily occur from noninactive channels.

Single semitendinosis muscle fibers dissected from the bullfrog *Rana catesbiana* were voltage clamped at 10°C using the vaseline gap procedure [3]. The Ringer's composition was 114 mM NaCl, 2 mM $CaCl_2$, 2.4 mM KCl and 5 mM Hepes/NaOH at pH 7.2. In addition 7.5 mM tetraethylammonium was used to block voltage-dependent K^+ channels. Fibers were cut in the end-pools in an

intracellular substitute containing 100 mM KF and 27 mM Hepes/NaOH at pH 7.4.

Schwarz et al. [4] have recently published results on myelinated nerve showing that phenobarbital induces slow recovery from sodium channel inactivation. I have made similar observations with voltage clamped semitendinosis muscle fibers using smaller doses of phenobarbital. These results are described and interpreted somewhat differently below.

The muscle membrane is clamped near its normal resting potential (about 90 mV) where 60—65% of the channels are not inactivated. At this same holding potential 500 μM phenobarbital blocks 31 ± 4% (mean ± S.E.) of the peak sodium current if channel block is assessed using an infrequently applied, 5 ms long step depolarization of 60 mV amplitude. However, if each depolarizing test pulse is preceded by a 45 ms long hyperpolarizing prepulse of 45 mV amplitude (to remove normal inactivation) then the blocking potency of this drug appears reduced. Now 500 μM phenobarbital blocks only 21 ± 2% of the peak sodium current.

Since the local anesthetic block of sodium channels is very state-dependent [1], it may be that barbiturates also bind channels in a state-dependent manner. For instance, if noninactivated channels (called resting channels below) show a much weaker affinity for phenobarbital than inactivated channels do then the hyperpolarizing prepulse could, by removing normal inactivation, also make the drug appear less potent. Schematically, such observations can be explained by the kind of kinetic scheme in Fig. 1 which allows for all possible transitions between closed states of sodium channels and includes a possible h-shift in the operation of drug altered channels. If $k_r/l_r < k_i/l_i$ then the slowed recovery from sodium inactivation reported by Schwarz et al. [4] during phenobarbital exposure can be reinterpreted as slow unbinding of phenobarbital from the resting channel state during the prepulse.

Such a kinetic scheme leads to an interesting quantification (and simplification) of these results with phenobarbital. First, assume that h-gating is kinetically much faster than drug blocking/unblocking steps. Then net closed channel blocking (k) can be represented in terms of the state-dependent component

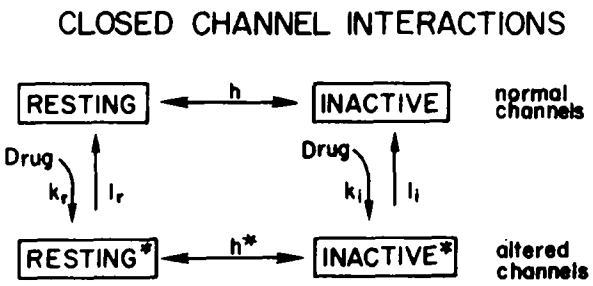

Fig. 1. Different sodium channel states may have different capabilities for drug interactions according to the modulated receptor hypothesis [1]. Closed channel (resting or inactive) transitions only are shown here. Inactivation gating represented by the Hodgkin-Huxley h variable and inactivation of drug-altered channels may be abnormal (h^*). Results with phenobarbital are described below which allow estimates of binding rate constants and show, for instance, that k_r is negligible for drug concentrations used here.

rate constants (Fig. 1):

$$k = k_r h_\infty + k_i(1 - h_\infty) \quad (1)$$

Net unblocking (l) can also be approximated by

$$l = l_r h^*_\infty + l_i(1 - h^*_\infty) \quad (2)$$

where h^*_∞, inactivation of the drug altered channels, may be enhanced [1,4]. If one could plot k against the inactivation variable h_∞ then estimates of the separate state-dependent binding parameters could be obtained. To do this, first determine h_∞ and peak sodium current levels for several different holding potentials before drug is added. Then measure the amount of sodium current block (b) using infrequent depolarizations at these same holding potentials during phenobarbital exposure. It will also be necessary to estimate at these holding potentials the time constants (T) governing recovery from prepulse train induced reductions in channel block (Fig. 2). Then

$$k = b/T \text{ and } l = (1 - b)/T \quad (3)$$

Measurements of T varied from 0.4 to 0.7 s for 250—500 μM phenobarbital concentrations. These time constants did not change appreciably for any given preparation for changes of up to 12 mV around the holding potential. However, time constants associated with block removal during large hyperpolarizing prepulses were typically 6-times faster (see also Ref. 4).

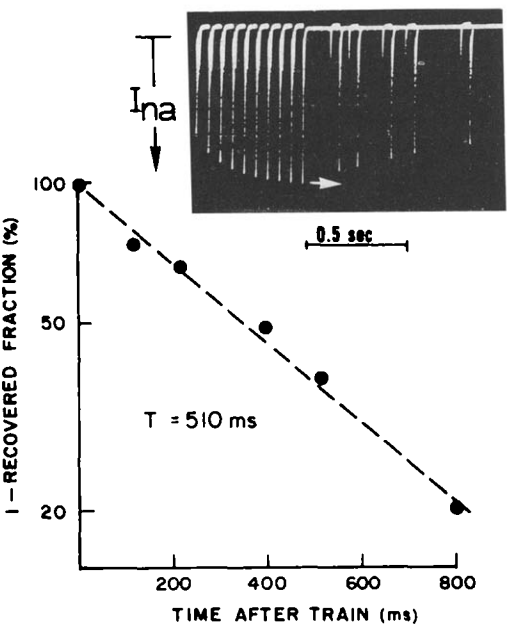

Fig. 2. Block recovery at the holding potential. Inset at top: peak sodium currents are first increased by a conditioning train of 45 mV, 45 ms long hyperpolarizations coupled with short depolarizations (60 mV, 5 ms long, used to monitor increases in peak current); recovery to original peak current level is then monitored at later times. A similar conditioning train was used before each and every recovery measurement (after arrow). Bottom: recovery plotted on semilogarithmic scale to establish recovery time constant (T) for 500 μM phenobarbital experiment illustrated here.

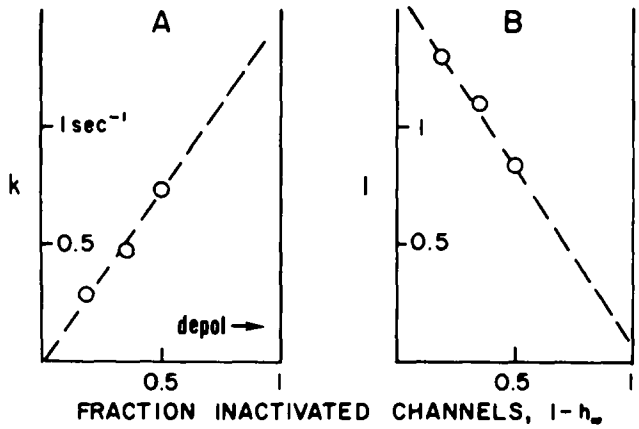

Fig. 3. Net blocking (k) and unblocking (l) rates are plotted for increasing levels of channel inactivation ($1 - h_\infty$) achieved by setting membrane at more depolarized holding potentials. Binding increases with depolarization (more inactivation) while unbinding decreases. 250 μM phenobarbital illustrated.

Fig. 3 illustrates an experiment where estimates of binding and unbinding were made at three inactivation levels. These results, examined using Eqn. 1, imply that only inactive channels are involved in the closed channel binding step. Primarily resting (noninactive) channels are implicated in the closed channel unbinding step. Similar results (at 2 h_∞ levels) were obtained pooling results of three additional experiments at 500 mM phenobarbital.

According to Eqn. 1, the Y intercepts in Fig. 3A should estimate values for the separate binding constants with k_i being 1.4 s^{-1} and k_r negligible in this case. Since inactivation of drug altered channels may be shifted the Y intercepts in Fig. 3B are not as useful. Separate estimates of l_r for these experiments (channel unblocking rate during hyperpolarizing prepulses) suggest l_r is about 8 s^{-1}. Both k_i and l_r estimates therefore satisfy the assumption made above that blocking/unblocking kinetics be much slower than h-gating which normally occurs at rates of 30—120 s^{-1} near the holding potential (Courtney, K.R., unpublished observations).

Thus, consideration of the modulated receptor hypothesis leads to an interesting interpretation regarding the effect of hyperpolarizing prepulses on sodium currents in the presence of phenobarbital. Such prepulses may, by favoring the removal of inactivation from the drug-altered fraction of sodium channels, reduce channel block. They would do so according to their amplitude and duration with longer, larger amplitude prepulses being capable of more completely removing phenobarbital block in the case illustrated here and elsewhere [4]. Since l_r, the unbinding rate constant for noninactivated, drug-altered channels may be as fast as 8 s^{-1} in this muscle fiber study even the conventional 50—100 ms long hyperpolarizing prepulses used to remove normal sodium channel inactivation in voltage clamp experiments may have a significant, additional channel unblocking effect in phenobarbital studies. Therefore sodium channel block by phenobarbital at normal holding potentials (h_∞ near 0.6) may be underestimated whenever prepulses are used to condition normal inactivation. Such a

circumstance also leads to an apparent shift in the inactivation (h_∞) curve in a manner dependent on the prepulse duration. Similar results regarding drug block of sodium channels may hold as well for other drugs that are small and thereby manifest fast escape rates from closed sodium channels [5]. For instance, lidocaine's potency in blocking muscle sodium currents is also somewhat reduced when monitored using hyperpolarizing prepulses before depolarizing pulses.

An estimate of the amount of inactivation present in drug altered channels near normal holding potential can be made by comparing l (estimated at holding potential) and l_r (estimated at a very hyperpolarized level) in a given experiment, since $l \geqslant h_\infty^* l_r$ (Eqn. 2). Such a calculation for phenobarbital experiments estimates $h_\infty^* \leqslant 0.1$ when $h_\infty = 0.6$. This amounts to an h shift for drug-altered channels of at least 20 mV. Schwarz et al. [4] attribute a similar h shift phenomenon observed in their studies to a selective slowing of the α_h process which governs recovery from sodium inactivation. The mechanism illustrated in Fig. 1, coupled with the results of Fig. 3B, suggests a distinctly different two-step recovery scheme ($I^* \rightarrow R^* \rightarrow R$) where l_r may be the rate limiting step.

Khodorov's 'slow inactivation' phenomenon [6], described for local anesthetics, is essentially contained in the scheme illustrated in Fig. 1. However, the results of Fig. 3B show that the recovery from slow inactivation (I^* state) may actually occur after the inactivation gate of drug altered channels is open rather than directly from the I^* state as modeled according to this hypothesis. The inactive state of the channel (h gate in place) appears to present a much higher affinity binding site to phenobarbital than the resting (noninactive) state does. This would occur if, for instance, there were two separate binding sites, one on the h gate and one on its channel latch, so that they were both available in the h gate-closed configuration only.

Enhanced binding to open sodium channels is also emphasized in explanations of frequency-dependent block of sodium channels by tertiary and (especially) quaternary local anesthetics. Barbiturates do not have a cationic drug form and so may not be able to access open sodium channels via this hydrophilic pathway [1]. Frequency-dependent block by phenobarbital [2] is more likely attributable to more blocking and less unblocking realized during depolarized (inactive) states of the sodium channel, with the kinetics of recovery between depolarizations (T) being slow enough to allow accumulation of frequency-dependent block at higher rates of depolarization.

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