Biochimica et Biophysica Acta, 597 (1980) 384—390

BBA 78667

PHENOBARBITAL INDUCES SLOW RECOVERY FROM SODIUM INACTIVATION AT THE NODAL MEMBRANE

J.R. SCHWARZ *, B. BROMM and G. OCHS

Institute of Physiology, University of Hamburg, Martinistr. 52, 2000 Hamburg 20 (F.R.G.)

(Received June 22nd, 1979)

Key words: Voltage clamp; Phenobarbital; Myelinated nerve fiber; (Na^+ channel, R. esculenta)

Summary

Voltage-clamp experiments were performed on single myelinated nerve fibres of *Rana esculenta* at 20°C in Ringer's solution and in solutions containing phenobarbital-sodium ([PB] $\leqslant$ 5 mM). The reduction of the sodium current under phenobarbital could be explained by an increase in the resting sodium inactivation; $h_\infty(E)$ was shifted towards more negative membrane potentials. The recovery from sodium inactivation proceeded with two time constants. The fast process could be described with the same time constant as in Ringer's solution, whereas the slow process had a time constant approx. 40 times larger. The slow process was also potential-dependent and could be described by $1/(0.025\alpha_h + \beta_h)$, where α_h and β_h denoted the rate constants in Ringer's solution. With the measured blockage of sodium channels by phenobarbital, both the shift of $h_\infty(E)$ and the slow recovery from sodium inactivation could be explained.

Introduction

At the peripheral nerve fibre, a reduction of the Na current and an inhibition of the K current under various barbiturates were observed [1—3]. Previously [4], the effect of phenobarbital on the Na permeability has been explained by an increase of the resting Na inactivation. The reduction of the Na current could be completely removed under large negative membrane potentials, yielding a shift of $h_\infty(E)$ to more negative membrane potentials. The main course of the recovery process proceeded at $E = -140$ mV with a time constant

* To whom correspondence should be addressed.

of approx. 7 ms. A comparable shift of $h_\infty(E)$ was also found in the presence of local anaesthetics as well as slow processes described as 'slow inactivation' [5] and 'use-dependent inhibition' [6—9]. The present experiments were performed in order to investigate the potential dependence of the time course of the removal from Na inactivation under phenobarbital. It will be demonstrated that the measured potential dependence of the slow recovery and the shift of $h_\infty(E)$ could be related to each other.

Methods

Voltage-clamp experiments were performed on single myelinated nerve fibres of *Rana esculenta* using the method of Dodge and Frankenhaeuser [10] in a modification described by Nonner [11]. The temperature of the solutions superfusing the node was adjusted to 20 °C. The neighbouring nodes were cut in isotonic KCl. Membrane potentials, E, were given as inside potential minus outside potential; outward currents were consequently positive. The resting potential was assumed to be $E = -70$ mV at $h_\infty(E) = 0.7$. Current densities were calculated from the measured potential drops across the internal resistance of the cut internode with a value of $Z_{ED} = 14\ \Omega \cdot \text{cm}^2$ [12].

The solutions used had the following compositions: Ringer's solution, 110 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$ and 5 mM Tris(hydroxymethyl)-aminomethane-HCl buffered at pH 7.3. Isotonic KCl, 117 mM KCl. The solutions containing phenobarbital-sodium (Merck-Schuchardt) were prepared before each experiment.

Results

The recovery from Na inactivation was investigated using the double-pulse procedure illustrated in Fig. 1. Conditioning prepulses, E_c, of variable duration, t, were followed by 2 ms test pulses to $E = -10$ mV. In both experiments under phenobarbital, the inward current for $t = 0$ was reduced and the recovery process was markedly slowed down. After a prepulse duration of 500 ms, the same peak current amplitude was attained as in Ringer's solution. The first part of both registrations is stretched in time scale, indicating the fast recovery process, whereas the latter part shows the further slow removal from Na inactivation.

The evaluation of the two time constants is illustrated in Fig. 2 for the conditioning prepulses $E_c = -130$ and -100 mV in Ringer's solution and using 2.5 mM phenobarbital. In Ringer's solution, a single exponential slope was observed in the semilogarithmic plot with the time constants 0.52 and 1.12 ms, respectively. The deviation of the first points of measurement for $E_c = -100$ mV from the straight line indicated the delay in recovery from inactivation reported by Chiu [13]. Under phenobarbital, two recovery processes could be separated as shown in the lower graphs of Fig. 2. The late phase of recovery was approximated by a straight line, yielding a large time constant, τ_{h2}; the subtraction of this line from the data points for short prepulse durations revealed a small time constant, τ_{h1}.

The time constants for recovery from Na inactivation in Ringer's solution

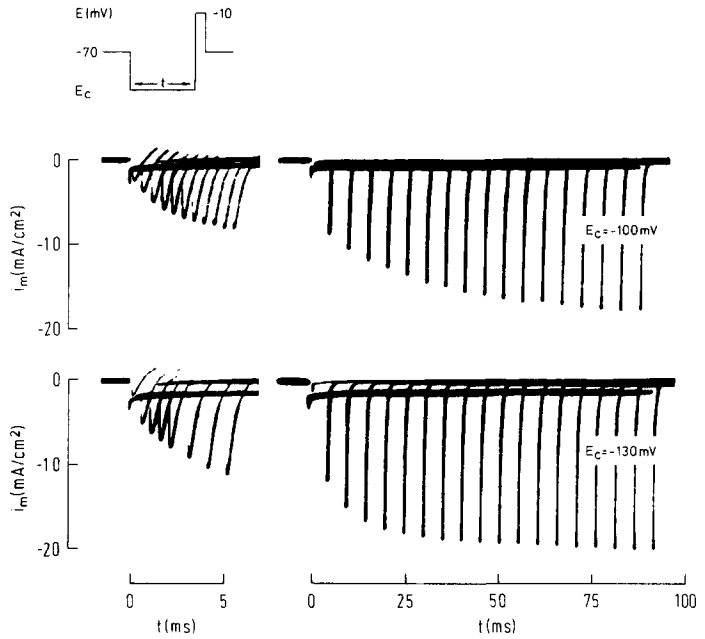

Fig. 1. Recovery from Na inactivation using 2.5 mM phenobarbital at different conditioning potentials. Inward currents during test pulses to —10 mV are recorded after conditioning pulses, E_c, of various duration, t, and superimposed photographically.

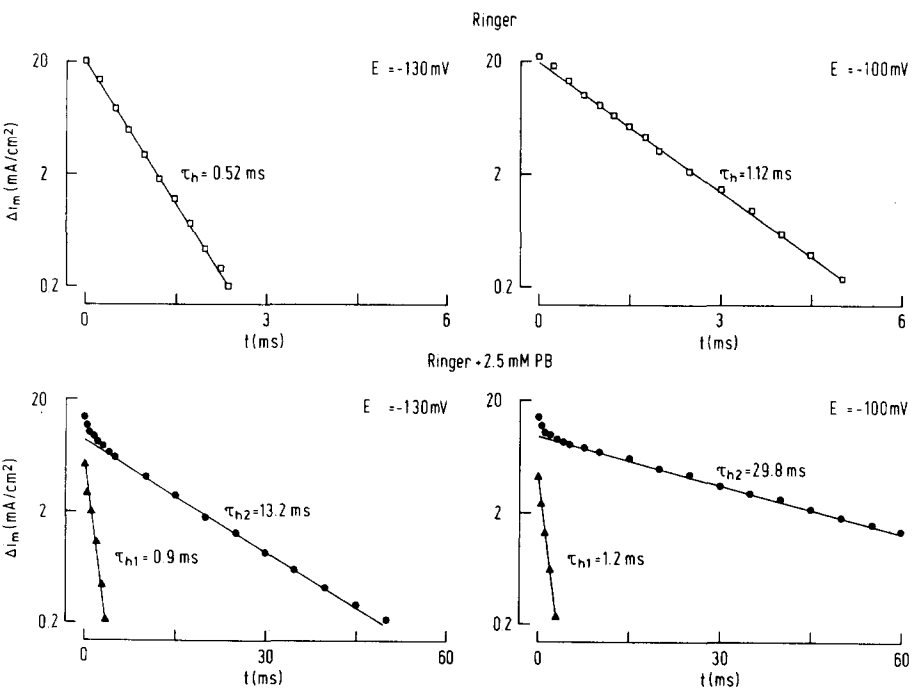

Fig. 2. Time constants of the recovery from inactivation in Ringer's solution and using 2.5 mM phenobarbital (PB) at two conditioning potentials. In Ringer's solution (upper graphs) the conditioning pulses were preceded by a potential step to —50 mV to fully inactivate the Na permeability. Using 2.5 mM phenobarbital (lower graphs) pulse program as shown in Fig. 1. The difference between the stationary and the actual current amplitude, Δi_m, is shown in semilogarithmic plots vs. duration of conditioning pulse, t. Ringer values (□) can be approximated by one straight line. Under phenobarbital the late decay of Δi_m yields τ_{h2}, the differences (▲) between the extrapolated late decay and the measured points are described by τ_{h1}. Experiment of Fig. 1.

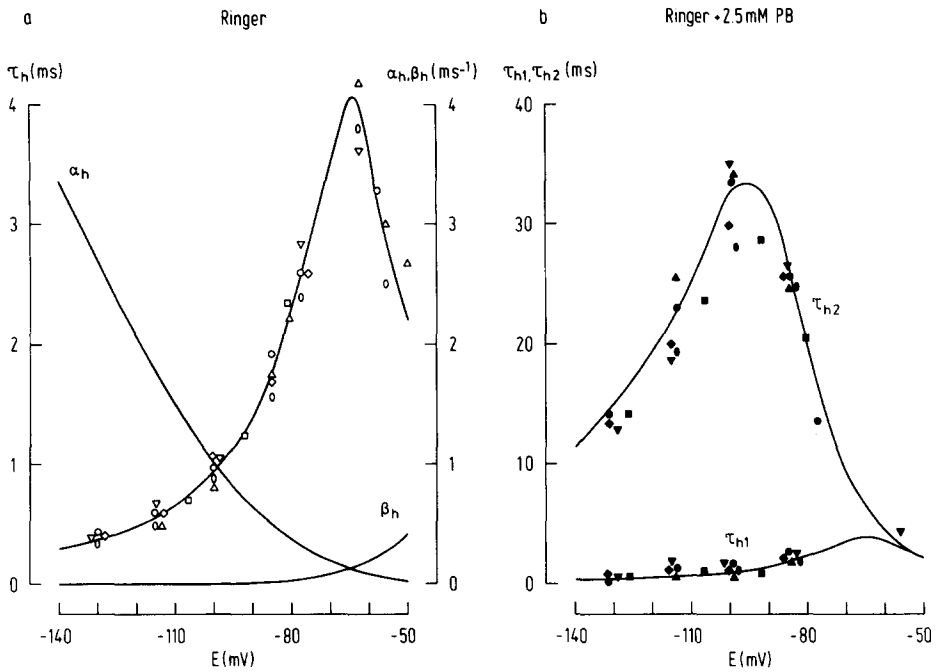

Fig. 3. Potential-dependence of time constants for recovery from Na inactivation in Ringer's solution and using 2.5 mM phenobarbital (PB). Different symbols refer to six different fibres: (a) The given exponential functions for α_h and β_h describe the potential-dependent τ_h in Ringer's solution (continuous curve), (b) The same α_h and β_h describe τ_{h1} under phenobarbital (note the different ordinate scales). The continuous curve for τ_{h2} is calculated with $0.025\alpha_h$.

and under phenobarbital were evaluated on six fibres in the potential range, $-130\ \text{mV} < E < -50\ \text{mV}$, and were plotted vs. membrane potential in Fig. 3. In these fibres, $h_\infty(E)$ was measured as well, using 500 ms prepulses. In Ringer's solution (Fig. 3a), τ_h attained maximum values of 4 ms near the resting potential. From the measured time constants, τ_h, and the $h_\infty(E)$ values, the rate constants, α_h and β_h, were calculated by using:

$$\alpha_h = h_\infty/\tau_h \text{ and } \beta_h = (1 - h_\infty)/\tau_h$$

The α_h and β_h values were approximated by exponential functions as given in Fig. 3a. The curve through the measuring points of τ_h was then calculated by using:

$$\tau_h(E) = 1/(\alpha_h + \beta_h)$$

To describe the results with phenobarbital (Fig. 3b), the ordinate units were enlarged 10-fold regarding the larger τ_{h2} values. The small time constant, τ_{h1}, was in fact the same as in Ringer's solution (Fig. 3a). τ_{h2} was also potential-dependent and could be fitted by:

$$\tau_{h2} = 1/(0.025\alpha_h + \beta_h)$$

with the same values for α_h and β_h as in Ringer's solution. Both time constants did not depend upon the concentration of the drug (1—5 mM) within the

accuracy of the measurements, while the shift of $h_\infty(E)$ obviously did.

Both effects of phenobarbital, the shift of $h_\infty(E)$ and the induction of a slow recovery from inactivation, could be referred to the assumption that the rate constant, α_h, was reduced. It was suggested that only a fraction of the Na channels, $h2$, exhibited slow inactivation, while the remaining part, $h1$, behaved as in Ringer's solution. Since the inactivation curve represents the fraction of resting, not inactivated pores, the measured $h_\infty(E)$ under phenobarbital should be the sum of the two contributions:

$$h_\infty = h1_\infty \cdot (1 - y_\infty) + h2_\infty \cdot y_\infty$$

y_∞ denotes the fraction of pores occupied by phenobarbital. With $y_\infty = 1$ and $0.025\alpha_h$, $h_\infty = h2_\infty = 0.025\alpha_h/(0.025\alpha_h + \beta_h)$. This equation yielded a shift of $h2_\infty$ by 26 mV towards more negative potentials compared to $h1_\infty(E)$. However, in the experiments using 2.5 mM phenobarbital a shift of 15.8 ± 1.7 mV (S.D., $N = 6$) was measured at $h_\infty = 0.5$. This discrepancy could be explained if only that fraction of pores occcupied by phenobarbital contributed to the shift of $h_\infty(E)$, whereas the fraction of unoccupied channels $(1 - y_\infty)$ did not and behaved as in Ringer's solution.

The fraction of Na channels affected by phenobarbital could be determined from the reduction of the peak Na current under different drug concentrations in relation to the controls in Ringer's solution, since there is a 1 : 1 reaction between phenobarbital molecules and receptor sites near the Na channel [4]. Under 2.5 mM phenobarbital, approx. 60% of the Na channels were occupied ($y_\infty = 0.6$). Only this fraction reacted with a 40-times smaller rate constant,

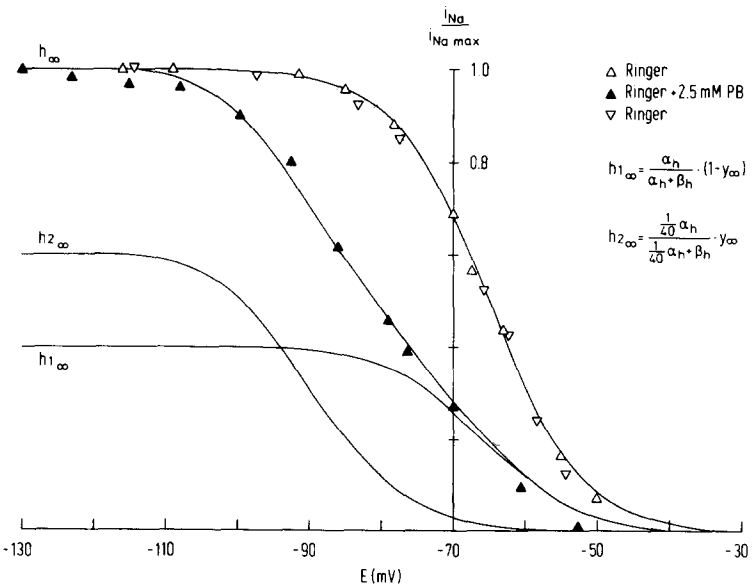

Fig. 4. The effect of 2.5 mM phenobarbital (PB) on the steady-state Na inactivation. $h_\infty(E)$ in Ringer's solution (△), under phenobarbital (▲), and after wash out (▽), measured by $i_{Na}/(i_{Na})_{max}$ (ordinate). Curve through open symbols calculated by $h_\infty = \alpha_h/(\alpha_h + \beta_h)$. $h1_\infty$ and $h2_\infty$ calculated from the equations given in the inset, with $y_\infty = 0.6$. Curve through the filled symbols represents the sum of $h1_\infty$ and $h2_\infty$. Motor nerve fibre; 20° C.

α_h, and shifted $h2_\infty(E)$ to more negative potentials. This is demonstrated in Fig. 4 by the curves labelled $h1_\infty$ and $h2_\infty$. The sum of both portions yielded the curve which approximates well the measured points of $h_\infty(E)$. This procedure was also applicable for experimental results under other phenobarbital concentrations. Using 1 and 5 mM phenobarbital, the measured shift was 8.5 and 21 mV, respectively, the calculated shifts amounted to 9.0 and 22.0 mV taking into account the measured respective reduction of the Na currents to 60% ($y_\infty = 0.4$) and 20% ($y_\infty = 0.8$).

Discussion

The experiments showed that the recovery from Na inactivation under phenobarbital could be described with two time constants. The fast process had the same time constant as in Ringer's solution; the slow process had an approx. 40-times larger time constant, and was potential-dependent as well. From the fraction of occupied Na channels (y_∞), the amount of the concentration-dependent shift under different phenobarbital concentrations could be predicted in agreement with the measurements. To illustrate these findings, the following model may be proposed. Consider h to be the relative portion of open (not inactivated) channels, $(1 - h1)$ the portion of the inactivated but unoccupied channels and $(1 - h2)$ the portion of closed (inactivated) channels occupied by phenobarbital. Thus, the following relations hold:

$$(1 - y_\infty) \cdot (1 - h1) \underset{\beta_h}{\overset{\alpha_h}{\rightleftharpoons}} (1 - y_\infty) \cdot h1$$

$$y_\infty \cdot (1 - h2) \underset{\beta_h}{\overset{\alpha'_h}{\rightleftharpoons}} y_\infty \cdot h2$$

with $h = h1 + h2$. The transitions from open to inactivated states are described by the relatively fast (Ringer-)process with the rate constants α_h and β_h; the slowed transition observed under phenobarbital takes place with $\alpha'_h = 0.025\alpha_h$ and with β_h; β_h is assumed to be equal for both transitions.

In experiments on lobster axons [5], squid axons [6] and frog myelinated nerve fibre [7], a reduction of the Na current and to a minor degree of the K current under barbiturates was observed. Our experiments showed that the increase in Na inactivation under phenobarbital reduced the Na current and could be completely removed. The slow recovery process and the shift of $h_\infty(E)$ could be interpreted as a decrease of $\alpha_h(E)$ by a factor of approx. 40 in the framework of the Hodgkin-Huxley formalism. These results have to be distinguished from other recent findings concerning Na inactivation. A slow inactivation component with time constants between 100 ms and 10 s was discovered by changing the holding potential under normal Ringer's solution [14,15]. Similar slow inactivation processes were found by altering the K^+ and Ca^{2+} concentrations [5,16]. Furthermore, slow recovery processes were found after application of local anaesthetics [8,9]. Depending on the degree of dissociation, these drugs are known to exhibit frequency-dependent reduction of the Na current. Very effective agents are the quarternary and tertiary amines which are positively-charged. Phenobarbital ($pK = 7.3$) is an anion:

it showed no use-dependence, like undissociated molecules, e.g., benzocaine at pH 7.3.

Acknowledgement

We wish to thank Professor Dr. W. Ulbricht for his comments on the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft, BR 310/13.

References

1 Blaustein, M.P. (1968) J. Gen. Physiol. 51, 293–307
2 Narahashi, T., Moore, J.W. and Poston, R.N. (1969) J. Neurobiol. 1, 3–22
3 Neuman, R.S. and Frank, G.B. (1977) Can. J. Physiol. Pharmacol. 55, 42–47
4 Schwarz, J.R. (1979) Eur. J. Pharmacol. 56, 51–60
5 Khodorov, B., Shishkova, L., Peganov, E. and Revenko, S. (1976) Biochim. Biophys. Acta 433, 409–435
6 Strichartz, G.R. (1973) J. Gen. Physiol. 62, 37–57
7 Courtney, K.R. (1975) J. Pharm. Exp. Ther. 195, 225–236
8 Hille, B. (1977) J. Gen. Physiol. 69, 497–515
9 Schwarz, W., Palade, P.T. and Hille, B. (1977) Biophys. J. 20, 343–368
10 Dodge, F.A. and Frankenhaeuser, B. (1959) J. Physiol. 148, 188–200
11 Nonner, W. (1969) Pflügers Arch. 309, 176–192
12 Frankenhaeuser, B. (1962) J. Physiol. 160, 40–45
13 Chiu, S.Y. (1977) J. Physiol. 273, 573–596
14 Fox, J.M. (1976) Biochim. Biophys. Acta 426, 232–244
15 Brismar, T. (1977) J. Physiol. 270, 283–297
16 Adelman, W.J. and Palti, Y. (1969) J. Gen. Physiol. 54, 589–606