Short communication

The Role of Inactivation in the Cumulative Blockage of Voltage-Dependent Sodium Channels by Local Anesthetics and Antiarrythmics

L. D. Zaborovskaya and B. I. Khodorov

Vishnevsky Surgery Institute, B. Serpukhovskaya 27, Moscow 113093, USSR

Previously published results (Khodorov 1973; Khodorov et al. 1974, 1976; Zaborovskaya 1976) showed that along with a decrease in the maximum sodium permeability ('tonic block') the tertiary amine local anesthetics (LA), procaine and trimecain cause a drastic slowing of Na^+ channel reactivation after membrane depolarization. This effect ("drug-induced slow Na inactivation") underlies a use-dependent Na^+ current (I_{Na}) inhibition during repetitive membrane depolarization. A detailed analysis of these data led Khodorov et al. (1976) to conclude that inactivation of Na^+ channels increased their affinity to LA which in turn resulted in stabilization of the inactivated channel conformation. The aim of the present work was to test this hypothesis using chloramine-T as a reagent capable of removing the ordinary ('fast') Na^+ channels inactivation (Wang 1983). We have examined the effect of chloramine T (CT) treatment of the nodal membrane on use-dependent (cumulative) inhibition of I_{Na} by lidocaine, tetracain, and the antiarrhythmic compounds N-propyl ajmaline (NPA) and KC 3791 (KC) (see Fig. 2*A*).

Experiments were carried out on single myelinated nerves of *Rana ridibunda* using the voltage clamp method (Dodge and Frankenhaeuser 1958). To block potassium currents, the internodes were cut in 114 mmol/l CsF solution. Both before and after CT treatment the node was superfused by control K-free Ringer solution containing (mmol/l): 112 NaCl; 2 $CaCl_2$; 2 $NaHCO_3$; Tris; pH 7.2.

After a short (5—15 min) exposure of the nodal membrane to 1—1.5 mmol/l CT, a certain fraction of Na channels irreversibly lost the ability to inactivate during 50—100 ms depolarizing clamp pulses. The remaining Na^+ channels retained the capability of a complete inactivation; however, the voltage dependence of this inactivation was shifted by about 20 mV towards more positive potentials (E). In this respect our results agree with those of Wang (1983). Application of 0.01 mmol/l tetracaine to the CT-pretreated node caused almost equal tonic

blocks of the peak (I_p) and steady-state (I_s) components of I_{Na}. However, during repetitive pulsing only I_p underwent a considerable cumulative inhibition; the noninactivating component of $I_{Na}(I_s)$ proved to be resistant to this type of block (Fig. 1). Qualitatively similar results have been obtained in experiments with 0.1—1.0 mmol/l lidocaine. This drug did not induce 'slow inactivation' or cumulative inhibition of I_s in CT-treated node of Ranvier.

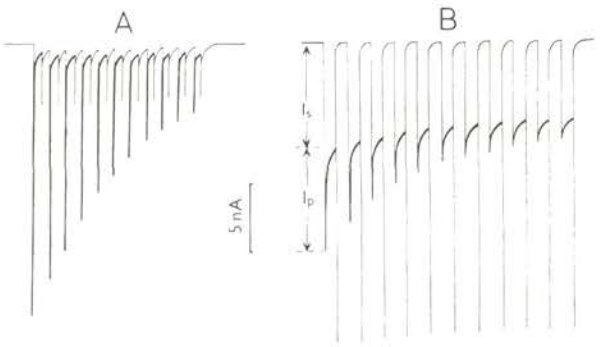

Fig. 1. Cumulative inhibition of sodium currents (I_{Na}) by 0.01 mmol/l tetracaine in normal (A) and chloramine-T (CT) pretreated (B) node of Ranvier. After 5-min treatment by CT (1.5 mmol/l) the node has been washed out with K-free Ringer solution. The frequency of pulsing 10 Hz, pulse duration 50 ms, $E = 0$ mV. Holding potential, $E_h = -100$ mV, the internodes were cut in 114 mmol/l CsF. *A*: fibre 29. 3. 84. Temperature 7 °C; *B*: fibre 2, 12. 4. 84. Temperature 10 °C.

Unlike lidocaine and tetracaine, NPA (Khodorov and Zaborovskaya 1983) and KC (present study) did not induce 'slow Na^+ channel inactivation'; the cumulative inhibition of I_{Na} caused by these drugs resulted from their interaction with open Na channels. Application of 10^{-4} mmol/l KC to the node of Ranvier pretreated with CT, produced approximately equal tonic inhibition of I_p and I_s. During repetitive pulsing both components of I_{Na} decreased markedly (Fig. 2). However, cumulative inhibition of I_p was somewhat more pronounced than that of I_s.

Qualitatively similar results have been obtained in studying the effect of 10^{-4} mmol/l NPA on CT-pretreated nodes of Ranvier.

Our results strongly support the notion, that lipid-soluble LA interact with inactivated Na^+ channels, thus inducing "slow inactivation" and cumulative blockage (Khodorov et al. 1976). By contrast, fast inactivation is not a prerequisite for cumulative inhibition of I_{Na} by cationic drugs interacting with open Na^+ channels (such as NPA or KC, used in this study, or QX314 or GEA968 employed in experiments of Shepley et al. 1983). Judging by changes of I_p and I_s caused by these drugs during repetitive pulsing (see Fig. 2), inactivation plays only an

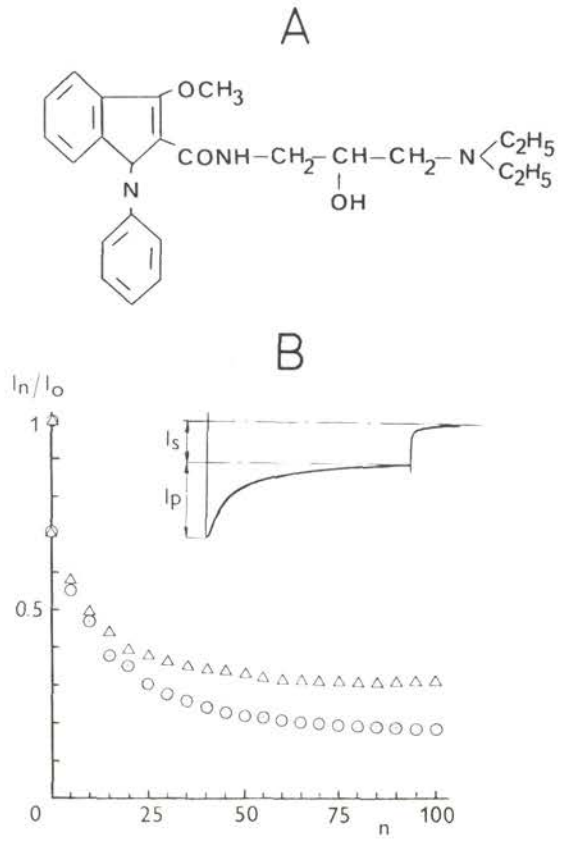

Fig. 2. Tonic and cumulative inhibition of I_{Na} by the antiarrhythmic KC 3791 (shown in A). Abscissa: number of the pulses (n); Ordinate; amplitude of I_{Na} relative units. The initial values of I_p and I_s before KC application were taken for unit. Repetitive pulsing (1 Hz) was turned on after 5-min exposure of the node in KC (0.1 mmol/l)-containing Ringer solution, which caused a tonic decrease in I_p and I_s value to 0.7. $E_h = 100$ mV, $E = 0$ mV. Pulse duration 40 ms. Fibre 19. 3. 84. Temperature 8 °C. Triangles: I_s; circles: I_p.

auxiliary role in the development of this type of cumulative blockage of Na channels. It is not easy to reconcile all these data with Hille's hypothesis (Hille 1977) of a common 'receptor' for all local anesthetics in the Na^+ channel.

Acknowledgement. We are very indebted to Dr. Weidner (Giulini Pharma) for providing N-propyl ajmaline and compound KC 3791 used in this study.

References

Dodge F., Frankenhaeuser B. (1958): Membrane currents in isolated frog nerve fibre under voltage clamp conditions. J. Physiol. (London) **143,** 76—90

Hille B. (1977): Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. J. Gen. Physiol. **69,** 497—515

Khodorov B. I. (1973): Slow sodium inactivation in the membrane of the node of Ranvier. In: Membrane Biophysics, Kaunas, pp. 621—629, (in Russian)

Khodorov B. I., Peganov E. M., Shiskova L. D. (1974): The effect of procaine and calcium ions on slow sodium inactivation in the membrane of Ranvier's node of frog.Bull. Exp. Biol. Med. **3,** 13—14 (in Russian)

Khodorov B. I., Shishkova L. D., Peganov E. M., Revenko S. V. (1976): Inhibition of sodium currents in frog Ranvier node treated with local anesthetics. Role of slow sodium inactivation. Biochim. Biophys. Acta. **433,** 409—435

Khodorov B. I., Zaborovskaya L. D. (1983): Blockade of sodium and potassium channels in the node of Ranvier by ajmaline and N-propyl ajmaline. Gen. Physiol. Biophys. **2,** 233—268

Shepley M. P., Strichartz G. R., Wang G. K. (1983): Local anesthetics block non-inactivating sodium channels in a use-dependent manner in amphibian myelinated axons. J. Physiol. (London) **341,** 62P

Wang G. K. (1983): Irreversible modification of sodium channel inactivation in toad myelinated nerve fibres by the oxydant chloramine-T. J. Physiol. (London) **346,** 127—142

Zaborovskaya L. D. (1976): A decrease in sodium permeability and slow sodium inactivation in the Ranvier node treated with trimecaine. Neirofisiologiya **8,** 418—425 (in Russian)

Received July 3, 1984 / Accepted August 8, 1984