Biochemical Pharmacology, Vol. 28, pp. 1451–1459.
Pergamon Press Ltd., 1979. Printed in Great Britain.

COMMENTARY

SOME ASPECTS OF THE PHARMACOLOGY OF SODIUM CHANNELS IN NERVE MEMBRANE. PROCESS OF INACTIVATION

B. KHODOROV
Vishnevsky Surgery Institute, Academy of Medical Science U.S.S.R. 113093, Moscow, U.S.S.R.

Sodium inactivation (SI) is the process postulated by Hodgkin and Huxley [1] to describe the decay of sodium permeability during a long-lasting membrane depolarization.

SI underliès many physiological events, being partially responsible for the falling phase of action potential, absolute and relative refractory states, accommodation, cathodal depression etc. (see [1–3] for reviews). Therefore many studies were devoted to the physicochemical nature of SI and its pharmacological properties. Some drugs used in these investigations proved to be very valuable as tools to study the molecular structure of Na channels.

The objective of the present paper is to discuss some aspects of the pharmacology of SI in nerve membrane.

1. PHARMACOLOGICAL SEPARATION OF ACTIVATION AND INACTIVATION IN SODIUM CHANNELS

There exist several chemicals which affect separately the activation and inactivation in sodium channels. Among them are different proteases, anemone and scorpion venoms (toxins), some ions and photodynamic sensitizers (dyes).

1.1. *Chemical removal of the fast sodium inactivation.* The external application of different proteases to the nerve membrane does not affect the properties of Na channels [4]. By contrast, the *internal* perfusion of the squid axon by pronase [5] or by its component—alkaline proteinase *b* [6], eliminates the fast SI (*h*-process, in Hodgkin–Huxley [1] formulation) without appreciable changes of Na activation. The *h*-process can be effectively destroyed also by the intraaxonal application of some other endopeptidases trypsin, chymotrypsin, papain [7, 8].

Unlike endopeptidase, the exopeptidases–carboxypeptidases *A* and *B* produce only the moderate suppression of Na^+ and K^+ conductances [7] with little or no change of SI [6–8].

From the analysis of all these data it was concluded that *h*-gate is proteinic in nature and that the polypeptide chain cleaved by proteases has to contain in its structure arginine (or lysine) as an important functional residue.

Developing this line of investigation, Eaton *et al.* [9] have shown that arginine-specific reagents-glyoxal and a condensed 2,3-butanedione-being applied internally are able to remove SI in squid giant axon. To test the possibility that positively charged arginine might actually be the blocking residue of *h*-gate these authors have compared the effects of internal application of various amino acids on I_{Na} in squid axon after a complete destruction of SI by pronase. Of nine amino acids tested only arginine showed a significant blocking ability. It was shown further that polypeptide polyglycyl-*N*-acylarginine induces the time-dependent inactivation-like inhibition of I_{Na} [9].

Recently Oxford *et al.* [9a] have found that along with arginine, a tyrosine residue appears to be involved in the inactivation gating structure of the sodium channel. Authors have compared the intraaxonal action of *N*-bromacetamide, destroying SI irreversibly, with the effects of several other group-specific protein reagents exhibiting overlapping reactivity spectra. *N*-Acetylamidozole, a tyrosine-specific reagent, proved to be the only other compound examined capable of partially mimicking *N*-bromacetamide.

Among the other chemicals producing a separate removal of SI by internal application to the squid giant axon membrane are NaF [10] and some common dyes [11]. The latter, e.g. Bengal Rose, sensitize the destruction of *h*-gate by a visible light illumination.

With the commonly used technique the internal side of the myelinated nerve fibre membrane is inaccessible to the proteolytic enzymes. It is possible, however, to destroy separately the *h*-gate in the Ranvier node with application of iodate ($-IO_3^-$) to the axoplasmic side of the nodal membrane (by letting it diffuse along the axis cylinder from the cut internode) [12, 13].

There exist, however, some chemicals which affect SI only upon *external* application to the nerve fibre.

The mini-protein toxin II (ATX-II) of the sea anemone (*Anemonia sulcata*) being applied to the frog Ranvier node [13, 14] or to crayfish giant axon [15] makes SI very slow and incomplete, while the activation kinetics remained unchanged. The intraaxonal application of these toxins is not followed by appreciable changes of sodium current.

The modification of sodium inactivation produced by some scorpion venoms or purified scorpion toxins are in many respects similar to those caused by anemone toxin ATX-II.

The drastic slowing down of the fast SI was observed under the external application of the crude venoms of *Leiurus quinquestriatus* (on amphibian myelinated fibres) [16], or *Buthus thamulus* (on squid giant axon) [17]. The same effects have been obtained in experiments with the purified scorpion toxins from *Androctonus australis* Hector (on crustacean giant

axon) [18] and *Buthus eupeus* (on frog node of Ranvier) [19, 20].

In addition to the deceleration of SI, scorpion venom (toxins) also induce very peculiar changes in the steady-state SI: the h curve (h_∞–fraction of channels free from SI at given potential) rises from a flat minimum to larger and larger values as the potential (E) become more positive [16, 20]. Very similar changes of the h_∞–E curve have been observed by Chandler and Meves [10] in NaF perfused squid axon and recently by Gillespie and Meves [20a] in squid axon treated externally by *Leiurus* venom. The explanation proposed is that in the modified channels the h-gate, which is closed by small depolarization, is forced open again by strong depolarization (the '*second activated state*') [10, 20a]. Gillispie and Meves [20a] assume that the increase of the time constant of SI under the action of *Leiurus* venom is only an apparent one and is, in reality, due to the appearance of the 'second activated state'. It is necessary, however, to remind that the increase of external Ca^{2+} eliminates the paradoxal rise of h_∞ at high E but does not affect the deceleration of SI in the node of Ranvier treated with *Leiurus* venom [21]. This means that scorpion toxins exert a dual effect on SI. The nature of these effects requires a further study. In our opinion in the scorpion venom-treated membrane two populations of modified Na channels do exist: (1) Na channels with slow and incomplete inactivation; and (2) Na channels with the inversed inactivation; their h-gate is closed at resting potential and becomes open during membrane depolarization.

According to the current views [22a] the ionic channels are composed of integral proteins, i.e. of amphipathic molecules with *hydrophobic* segments deeply buried in the lipid interior of the membrane, and *hydrophilic* segments protruding out of membrane plane into the water phase. Judging by the date discussed above, the 'inactivation mechanism resides in a large protein subunit which spans the membrane' [13]. The internal hydrophylic segment of this subunit seems to act as h-gate itself while the outer edge of the subunit bears the receptor groups for anemone and scorpion toxins. Some of these groups are Ca^{2+} sensitive [21, 22]. The destruction of h-gate by proteases [8], bromacetamide [9a] or iodate [13] does not remove the slow ('ultra-slow' [23]) SI which is characterized by time constants of order of tens of seconds and even minutes [23–25].

Therefore it is reasonable to propose that the hydrophobic part of the inactivation subunit, disposed in the lipid matrix of the membrane, is involved in the process of slow SI.

1.2. *Selective modification of sodium activation.* Unlike *Leiurus* and other above-mentioned scorpions toxins the *Centruroides sculpturatus* scorpion venom induces a selective modification of sodium activation [26]. This modification is manifested after the end of depolarizing pulse as a temporary shift of the voltage dependence of sodium activation by 40–50 mV to more negative potentials (Fig. 1). As a result of such a shift the inward sodium current continues to flow through the activated Na channels during hundreds of milliseconds upon membrane repolarization. The kinetics of sodium activation and inactivation are not changed under the action of this drug. Figure 1 shows the calculated behaviour of sodium current and underlying changes of activation (m^3) and inactivation (h) factors

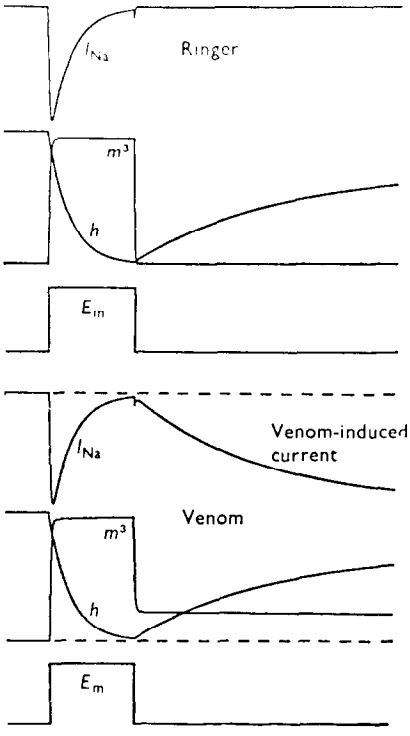


Fig. 1. The calculated time course of Na activation (m^3) and inactivation (h) underlying Na currents in the node of Ranvier before (Ringer) and after application of *Centruroides sculpturatus* scorpion venom. For simulation of venom currents the m_∞–E curve was shifted by 50 mV to more negative voltages [26].

in normal and venom treated model membrane. One can see that in the poisoned node after the end of a depolarizing pulse the activation parameter m^3 remains increased for about 200 msec allowing I_{Na} to rise as recovery from inactivation occurs. From the voltage- and time-dependence of channels modification it was concluded that the drug interacts only with the open Na channel. However the fraction of modified channels continues to rise for depolarization larger than that at which all the channel become open. This means that the drug-receptor reaction depends directly upon the field across the membrane.

All the data discussed above show that both activation and inactivation processes are chemically separable, and thus can be thought of as being associated with different structural identities of the channel.

2. INTERACTION OF ACTIVATION AND INACTIVATION PROCESSES

Chemical separability of activation and inactivation in sodium channels does not mean yet that these processes are completely independent, as was postulated for the sake of mathematical simplicity by Hodgkin and Huxley [1]. During the last few years data have been obtained favoring the assumption [27, 28] of coupling of sodium activation and inactivation processes [29–31]. Here we would like to discuss some pharmacological aspects of this problem.

2.1. *Simultaneous modification of activation and*

inactivation induced by batrachotoxin and aconitine. Batrachotoxin (BTX) and aconitine (AC) belong to the chemicals inducing a steady depolarization of nerve membrane due to the increase of its resting sodium permeability (P_{Na}) [32]. The voltage–clamp experiments carried out on frog Ranvier nodes showed that both BTX [33–37] and AC [38–40] cause a dramatic alteration of the gating machinery [33–35] and of selectivity [36, 37, 39, 40] of Na channels. The latter lose the ability to inactivate, and simultaneously drastic changes of activation process occur. The voltage-dependence of activation is shifted by about 50 mV to more negative voltages, so that a considerable sodium current flows at the resting potential (−70 mV). The activation kinetics becomes purely exponential (instead of being sigmoid) (Fig. 2) and the time constant of activation is increased 5–10-fold under the action of BTX and about two-fold in the case of AC action. The ionic selectivity of AC-modified [39, 40] and BTX-modified [34, 36, 37] Na channels is greatly decreased. So under the action of BTX Na channels become measureably permeant to Rb^+, Cs^+, Ca^{2+} and even to methylammonium. The relative permeability of these channels to all the permeant cations is increased, especially to NH^+ and Tl^+.

The process of membrane modification (Na channels recruitment) under the action of BTX (10^{-5} M) or AC (10^{-5} M) is relatively slow (min) but it can be greatly accelerated using strong repetitive depolarizing pulses [36, 40, 41]. A detailed study of this effect in BTX-poisoned Ranvier mode led to the conclusion that BTX interacts with Na channels, only (or mainly) when both activation and inactivation gates are in the open position [41].

The use of the repetitive membrane depolarization allowed to study the dose–response relation and to infer that each channel receptor interacts only with one molecule of BTX [42]. This conclusion is of great importance since it indicates that all the changes of sodium activation, inactivation and ionic selectivity caused by BTX result from its interaction with a single channel receptor.

It is very tempting to assume that this receptor is linked allosterically to all the principal subunits of sodium channel, gates, sensors, selectivity filter, and plays therefore a key role in their normal functional interaction [36]. Binding of BTX or AC to the receptor alters this normal subunits interaction and causes the above-mentioned modification of channels properties.

According to Catterall [43, 44] alkaloid varatridine—another modificator of Na channels—interacts with the same receptor as BTX and AC. Yohimbine [45] and some tertiary (procaine, trimecaine) and quaternary (QX-572) local anesthetics [34, 36] seem to compete with BTX for a common receptor (or common sites of this receptor).

Some indirect data show that BTX-receptor is protein in nature [32] and suggest its localization in the hydrophobic part of the channel [45a].

The poor reversibility of the effects of BTX and AC should make it possible to extract and identify the Na channel structure by means of labelled drugs.

2.2 *Inactivation of sodium gating current.* It is established at present that the activation of sodium channels is closely associated with the intramembrane charge movement revealing, in the form of the so-called gating current, I_g (see [46] for a review). Many attempts were made to detect the *independent* charge movement related to the inactivation of Na channels. However, quite unexpectedly for the prediction of Hodgkin–Huxley model [1], it was found that sodium permeability inactivation is associated with "immobilization" (or neutralization) of the gating particles responsible for the opening (activation) of Na channels [47].

In the experiments on the squid giant axons [47] and myelinated nerve fibres [48] it has been shown that a depolarizing conditioning prepulse which inactivates I_{Na}, diminishes I_g as well. Judging from recent data [48] the net I_g includes at least two different components,

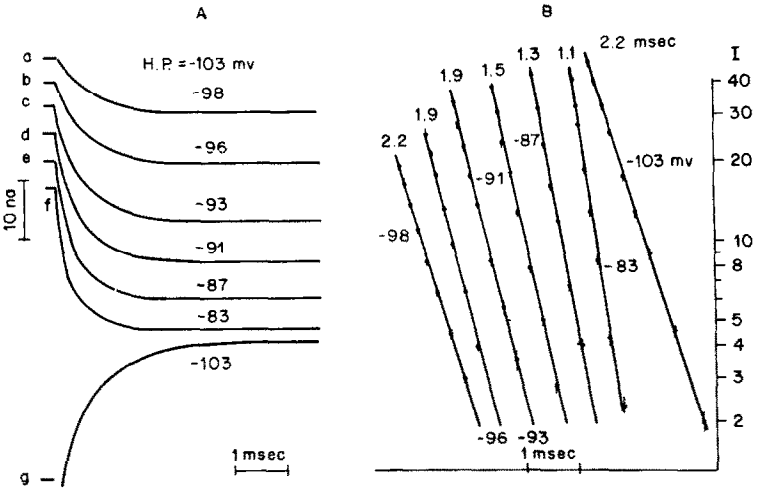


Fig. 2. Effect of batrachotoxin on Na activation kinetics. A: (a)–(f), Na currents associated with depolarizing steps from holding potential −103 mV to various potentials (indicated at each trace), (g) Na current on return after 5 msec depolarizing pulse to −103 mV. B: semilogarithmic plot of the time course of I_{Na}. Numbers above each curve are the time constants (τ_m) of activation in msec. Leakage current is not subtracted; 20° [36].

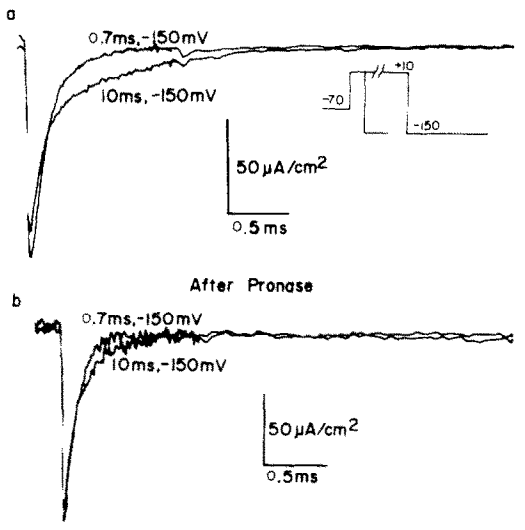


Fig. 3. Effect of the enzymatic removal of Na inactivation on the off-response in the giant axon of the squid. (a) The voltage sequence: $-70 \rightarrow +10 \rightarrow -150$ mV. The depolarizing pulse duration 0.7 and 10 msec. (b) Another axon after pronase had destroyed 70 per cent of inactivation. The voltage sequence: $-80 \rightarrow +20 \rightarrow -150$ mV. External solution (mM): 90 Na, 50 Ca, 40 Mg + TTX. Internal solution: 190 TEA, 10 Na, 8° [47].

one of which can be completely inactivated with a short (5–10 msec) depolarizing prepulse ("fast inactivating components"). Only the long-lasting (min) membrane depolarization is followed by complete inactivation of the net I_g [25].

The other aspect of the fast I_g inactivation is a diminishing of the reverse gating charge movement ("off-response") at the end of the depolarizing pulse with a duration long enough to induce the inactivation of sodium permeability [19, 47, 49, 50]. Figure 3 (top) demonstrates this phenomenon. One can see that the amount of charges (Q_{off}) transferred at the end of 10 msec depolarizing pulse is clearly less than at the end of the 0.7 msec pulse. We also see the splitting of the I_g-tail into two fast—and slow components. The interpretation of these changes of the "off-response", proposed by Armstrong and Bezanilla [47] is that about two-third of charges "is immobilized by inactivation and moves back to rest too slowly to produce a measurable current" (p. 302).

Pronase in the squid axon (Fig. 3) and *Buthus eupeus* scorpion toxin in the frog node of Ranvier [19] remove all these changes of the off-response. Therefore it is reasonable to assume that the inactivation of I_g results from interaction (possibly electrostatic in nature) of the activating gating particles with an "inactivation subunit" of Na channel. The following kinetic scheme may be proposed to describe these events quantitatively:

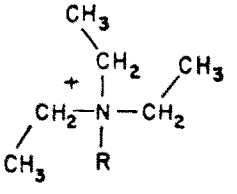
$$A \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} B \underset{\delta}{\overset{F}{\rightleftharpoons}} C$$

Here A, B and C are the resting, activated and fast inactivated states of the hypothetical gating particles, respectively; α and β—voltage-dependent rate constants of activation process; γ and δ—the association and dissociation (respectively) rate constants of the reaction between gating particles and F-receptor (h-gate) responsible for the fast inactivation. γ and $\delta \ll \alpha$ and β.

We have mentioned above that proteases do not remove the ultra slow SI [8]. Therefore it is very tempting to propose that along with the F-receptor, associated with the fast inactivation of I_g, another receptor (S) exists which is localized in the hydrophobic part of the channel and is able to interact with some (or with certain) gating particles and "immobilize" them for a long time. This question is discussed in a more detailed form elsewhere [47a].

3. SODIUM INACTIVATION AND THE INTERNAL BLOCKADE OF SODIUM CHANNELS WITH QUATERNARY AMINE COMPOUNDS(QA)

In the experiments on the internally perfused squid giant axons it was discovered that the enzymatic destruction of the h-gate renders the Na channel sensitive to the blocking action of internally applied tetraethylammonium (TEA) or some of its derivatives shown in Fig. 4. These compounds are specific blockers of potas-



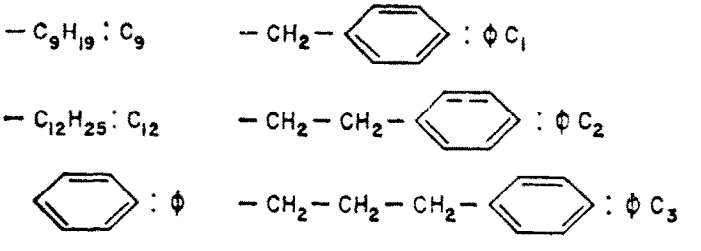
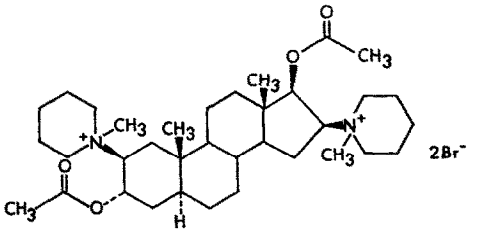
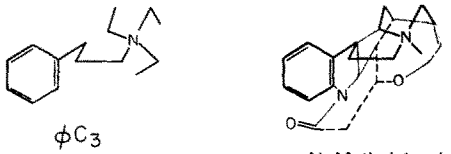


Fig. 4. TEA and its derivatives inducing a time-dependent blockade of K channels. The R groups are shown with abbreviations used to denote them [51].



Pancuronium Bromide



ϕC_3
(Armstrong,1971)

N-Methylstrychnine

Fig. 5. Chemical structure of pancuronium (PC) and of quaternary analog of strychnine (NMS). The latter is compared with K channels blocker compound ϕC_3. Common structure is drawn in more heavily in NMS [54, 55].

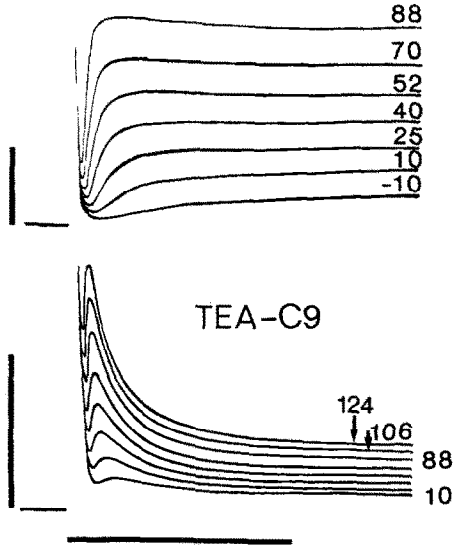


Fig. 6. Effects of TEA derivative C_9 (see Fig. 4) on outward I_{Na} in a squid giant axon after destruction of h gate. Pulse duration 1.5 msec. In A, maximum outward current for $E = 88$ mV was 5.4 mA/cm², and 2.3 mA/cm² in B. Fibre treated internally with proteases contained in pronase. A, internal solution: 300 mM NaF + 400 mM sucrose; external solution was K^+- and Na^+-free saline. 9.5°. B, after internal application of C_9 at 0.15 mM. Potentials (in mV) are indicated at each current trace. [6].

sium currents but do not affect intact sodium channels [51, 52].

The simplest way to explain this effect is to suppose that h-gate in the intact sodium channel hinders the entrance of TEA^+ in the internal channel mouth purely sterically.

It is known, however, that sodium currents in the normal axons can be effectively inhibited by intraaxonal application of quaternary amine-local anesthetics [53], quaternary derivative of strychnine (NMS) [54], bis-quaternary myorelaxant pancuronium [55] and some other quaternary compounds [56] which also may be considered as derivatives of TEA (Fig. 5).

Therefore it seems to us very probable that "h-gate" bearing the positive charge [69, 47], hinders the stabilization of TEA cation in the internal channel mouth on account of the electrostatic charge repulsion. Removal of the inactivation gate converts TEA into an Na channel blocker.

Of special interest is the time course of I_{Na} inhibition caused by different quaternary ammonium compounds. As has been mentioned above, the compounds shown in Fig. 4 (from now on referred to collectively as QA-I) become effective only after destruction of the inactivation gate. Then inhibition is time- and voltage-dependent and qualitatively similar to the inhibition of potassium currents produced by these compounds (Fig. 6).

The model proposed by Armstrong [51, 57] for the potassium channels blockade is:

$$\text{Resting channels} \underset{\text{kinetics}}{\overset{\text{Hodgkin–Huxley}}{\rightleftharpoons}} \text{Open channels} \underset{l}{\overset{\text{drug } k}{\rightleftharpoons}} \text{Blocked channels}.$$

The drug molecule enters the internal channel mouth after the opening of the activation gate and binds to the corresponding receptor sites with the association and dissociation rate constants k and l, respectively. One of these sites (A) seems to be anionic (interaction with the cationic head of QA) and the other (H), hydrophobic in nature.

The hydrophobic interaction of the hydrocarbon and/or aromatic "tail" of the QA molecule with H-site stabilizes QA in the channel mouth (on account of the decreasing of l-rate constant). During a strong depolarizing pulse, the fraction of blocked channels (B) increases to some equilibrium value $B \approx 1/(k + l)$.

Unlike the QA-I which are able to plug only the Na channels devoid of h-gate, the quaternary amine local anesthetics (QX-314, QX-572, QT), quaternary analog of strychine (NMS), and pancuronium (from now on referred to collectively as QA-II) block effectively both intact and devoid of SI Na channels [53–56, 58, 59].

We cannot discuss here the specific changes of the time course of I_{Na} induced by QA-II in the intact membrane [54, 55]. It is necessary only to emphasize that all these changes are predicted by a model similar to that presented above, if one assumes that QA enters the channel only when both activation and inactivation gates are open, and that the activation gate cannot be closed upon the membrane repolarization until a big QA molecule leaves the channel [54, 55].

For what reason can QA-II block both the intact and the deprived of inactivation Na channels while QA-I are able to plug only the latter?

The drugs belonging to QA-II group differ greatly in their chemical structure (see Figs. 5 and 7); however they have some common features: in contrast to the QA-I provided with purely hydrophobic "tails", the QA-II molecules have —CO and —NH groups included in the tails.

Since —CO and —NH groups can form hydrogen bonds, it is logical to assume that the hydrogen bonds formation between QA-II 'tail' and hydrophilic segment of the inactivation subunit (*h*-gate) in the intact sodium channel is prerequisite for stabilization of QA in the normal channel mouth. Only after enzymatic destruction of the *h*-gate does the purely hydrophobic interaction become sufficient for such a stabilization of the QA-channel complex.

Thus we can conclude that the chemoreceptor existing in the axoplasmic end of the intact sodium channel includes along with the anionic (A) and hydrophobic (H) sites also the sites which are able to form hydrogen bonds with —CO and —NH groups. This makes the functional structure of the receptor in question in some respects similar to the structure of cholinoreceptors of different excitable membranes: it is well known that cholinoreceptor also includes anionic, hydrophobic and "esterophilic groups" [60].

Block of the intact Na channels by quaternary amine compounds in myelinated nerve fibre [53, 54] and in the squid giant axon [56, 58, 59] is voltage and frequency dependent. Many short (1–5 msec) depolarizing pulses produce much more inhibition than a single long (1 sec) depolarizing step.

Destruction of the inactivation gate in squid giant axon by proteases abolishes the use-dependent block caused by QX-314, QX-222, 9-amino-acridine, and *N*-methylstrichnine [59, 59a, 59b]. The same effect was obtained in the Ranvier node treated with QX-572 after removal of SI by BTX [36].

These results suggest that the *h*-gate hinders the exist of QX from the blocking site in the channel mouth. The use-dependent inhibition disappears very slowly (several minutes) however, in many cases the restorative process can be greatly accelerated using the train of low amplitude depolarizing pulses preceded by strong hyperpolarizing prepulses [53]. These data suggest that a QA-II molecule entering the internal channel mouth "retains" the *h*-gate in the closed position in spite of membrane repolarization. Strong hyperpolarizing prepulses are necessary to open these closed *h*-gates.

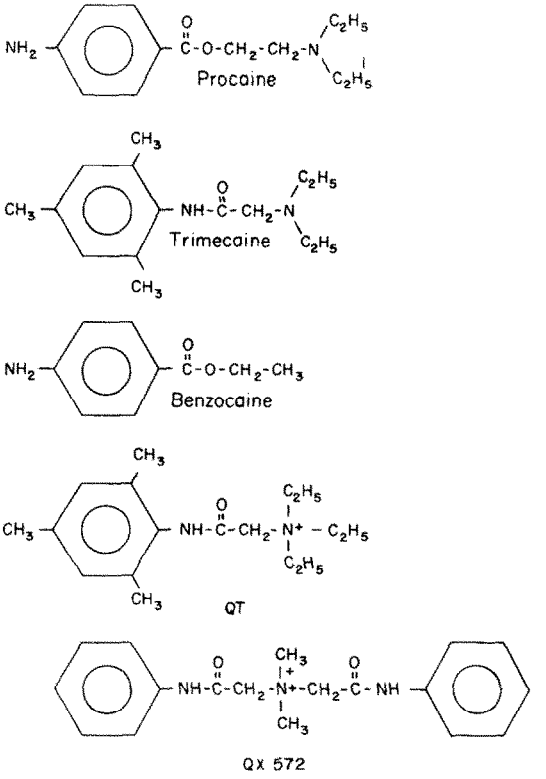


Fig. 7. Chemical structure of local anesthetics. QT—quaternary derivative of trimecaine; QX-572—quaternary derivative of lidocaine.

It is noteworthy that some of QA-II compounds, e.g. quinidine methiodide [56] are able to block Na channels in the use-dependent manner even after enzymatic (pronase treatment) destruction of *h*-gate. This apparently means that such drugs are able to bind to the anionic and hydrophobic sites of the compound receptor in the internal channel mouth so firmly that after the end of a depolarizing pulse they remain in the blocking position even "without the help" of the inactivation gate.

In conclusion of this section it is necessary to mention that not only the permanently charged quaternary amine compounds but also their tertiary analogs in protonated form are able to plug the open axoplasmic end of the sodium channel and cause the use-dependence inhibition of I_{Na} both in myelinated fibre [63–65] and in squid giant axon [59b]. Destruction of *h*-gate by pronase abolishes the use-dependent block caused by tetracaine and etidocaine only partially [59b].

4. MODIFICATION OF SODIUM INACTIVATION UNDER THE ACTION OF LOCAL ANESTHETICS

Tertiary amine local anesthetics (LA)—procaine, trimecaine, lidocaine (Fig. 7)—commonly used in clinical practice, exert a dual inhibitory effect on sodium conducting system: they reduce the maximum of sodium permeability, $\bar{P}_{Na}$, and modify the properties of sodium inactivation.

The decrease of $\bar{P}_{Na}$ is due to a voltage-independent blockage of Na channels. It cannot be removed by membrane hyperpolarization, or by changes of external Ca^{2+} concentration [61–63], but is very sensitive to external pH: its increasing over the range 6–10 greatly enhances [61, 63] and accelerates [64, 65] the LA-induced reduction of $\bar{P}_{Na}$. From these data it was inferred that the neutral form of LA must diffuse in the lipid membrane matrix to reach the site of action [65]. The latter can be considered as a receptor (R_I) with a certain stereospecificity, since it can discriminate between tertiary enantiomers RAC 109 I and II applied from outside of the Ranvier node [64]. Judging from the dose–response curve, one molecule of LA must bind the receptor to block one channel [61, 63].

Some data obtained on myelinated nerve suggest that R_I is localized in the internal channel mouth, and that it is common for both tertiary and quaternary amines compounds [65]: (1). The difference in the blocking potency of the quaternary RAC 421 I and II applied inside (through the cut internode of the myelinated fibre) is similar to that of the tertiary RAC 109 I and II applied from outside. This means that the corresponding receptors have similar sterospecificity [64]; (2). BTX causes about 10-fold reduction of the blocking action (decrease of $\bar{P}_{Na}$) of both tertiary (procaine, trimecaine) and quaternary (QX-572) local anesthetics [34, 36]. It is necessary to emphasize that not

only tertiary and quaternary LA anesthetics, but also neutral benzocaine is able to decrease $\bar{P}_{Na}$. The equilibrium dissociation constant of the benzocaine–channel reaction (0.4 mM) [61, 69] is close to those for procaine (0.2 mM) [61] and trimecaine (0.3 mM) [63]. However BTX does not antagonize the blocking action of benzocaine [34, 36].

To explain these data in frame of the notion of a common receptor responsible for the decrease of $\bar{P}_{Na}$ it is necessary to assume that benzocaine binds to the hydrophobic site (H) of the compound chemoreceptor in the internal channel mouth, and that this site is not involved in the BTX-channel interaction.

The changes of SI caused by tertiary LA are manifested as follows: (1) the voltage dependence of the steady state fast inactivation (h_∞–E curve, where h_∞ is a fraction of Na channels free from inactivation at potential E) is shifted to more negative voltages [65]; (2). The time constant of sodium reactivation (opening of the previously closed h-gate) is increased a hundred times [62, 63, 65].

The detailed study of these effects on myelinated nerve [62, 63, 65] and skeletal muscle [67] allowed us to assume that the changes of SI caused by LA are due to their interaction with the inactivated Na channels [63].

This interaction hinders in some way the opening of h-gates until the drug–channel complex becomes dissociated.

The kinetic scheme to describe this model is

$$O \underset{\alpha_h}{\overset{\beta_h}{\rightleftharpoons}} C \underset{l}{\overset{k}{\rightleftharpoons}} C^*$$

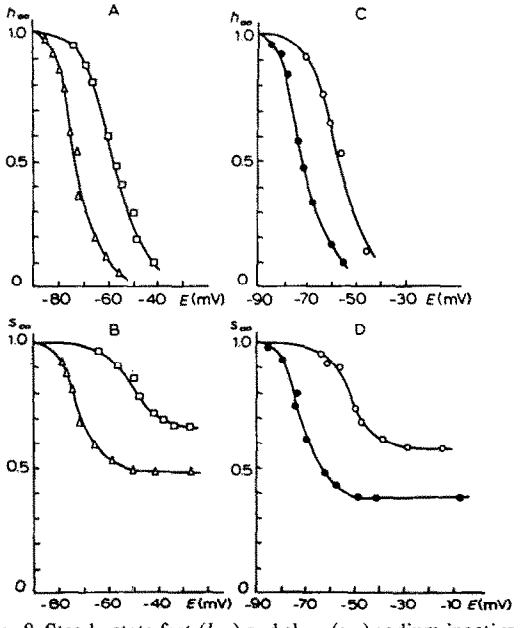


Fig. 8. Steady-state fast (h_∞) and slow (s_∞) sodium inactivation related to membrane potential at normal and increased external Ca^{2+} concentration. A, B: node in Ringer solution containing 10 mM KCl (triangles) and 10 mM KCl + 20 mM Ca^{2+} (squares). C, D: node in Ringer solution with 0.054 mM procaine (filled circles) and 0.054 mM procaine + 20 mM Ca^{2+} (open circles) [63].

Here O, C and C^* are the states of h-gate: open (resting channel), closed (inactivated channel) and closed–modified, respectively. α_h and β_h are the voltage-dependent Hodgkin–Huxley rate constants of the fast inactivation process. k is the forward rate constant of the drug-inactivated channel reaction; its value increases with increasing of LA concentration. l is the backward rate constant which is independent of concentration of LA, amplitude or duration of depolarizing pulse. Both k and l are 100 times smaller than β_h and α_h, therefore the $C \rightleftharpoons C^*$ transition is slow. This process can be labelled tentatively as *slow drug-induced SI*. Its time constants are of the order of several hundreds of milliseconds.

The kinetics of $C \rightleftharpoons C^*$ transition is very sensitive to the external pH: its increasing from 5.6 to 8 is followed by increase both of k and l, (i.e. accelerates the development and removal of slow inactivation).

The model under consideration describes well all the voltage-, time- and frequency-dependent effects of procaine, trimecaine [62, 63] and lidocaine [66]. Of great interest is the discovery of Schwarz *et al.* [67] that in contrast to external pH, internal pH changes (6.5–8.4) do not affect the time constants of $C \rightleftharpoons C^*$ transition.

Fig. 8 C and D compares the steady-state dependence of the fractions of Na channels free from fast (h_∞) and slow (s_∞) inactivation on the membrane potential in the procaine-treated Ranvier node. The s_∞–E curve has a shape quite similar to h_∞–E curve, however the slow modification of sodium inactivation is not 100 per cent; s_∞ decreases to some non-zero level s_∞ . The important point is the s_∞ reaches this s_∞ level just in the same potential region where h_∞ becomes close to zero. The s_∞ level decreases with increasing drug concentration. The dose–response relation of these effects of LA could be fitted by assuming one-to-one reaction between LA molecule and receptor (R_2) of Na channel with equilibrium constant of order of 0.02 mM. Added divalent cations (Ca^{2+} or Ni^{2}) shift the steady-state slow SI curve (s_∞–E) to less negative potentials and simultaneously reduce SI at the saturation level (Fig. 8C, D).

Unlike tertiary LA, benzocaine and quaternary anesthetics (QT, QX-572) are not able to induce a slow SI [63, 66, 67].

The localization of the receptor responsible for the drug-induced SI is a point of controversy.

Hille [65] does not differentiate R_1 and R_2 receptors. According to his "modulated-receptor hypothesis" there exists a single receptor in the internal mouth of Na channel for the different drug types. "Any drug in channel increases the probability of closing the inactivation gate" (p. 497), i.e. the probability of state C^* (Fig. 9). The model predicts a strong dependence of blocking potency of LA on the state of the inactivation gate: "When h-gate is open, binding to the receptor is not very firm, but when the gate is closed the receptor is modified and the binding is stronger" (p. 510). However, the experiments have shown that the elimination of SI by BTX does not decrease the blocking action of benzocaine [34, 36].

Hille's model also does not explain a 10-fold difference of the equilibrium dissociation constants obtained in studying the effects of trimecaine on $\bar{P}_{Na}$ and on slow inactivation (s_∞^{min}) [63].

Slow inactivation induced by LA phenomenologi-

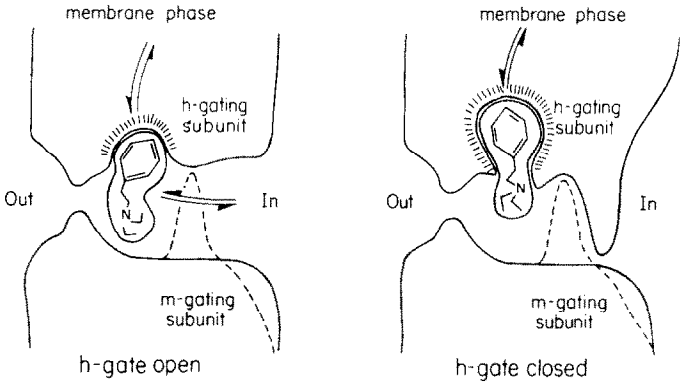


Fig. 9. Diagram of a local anesthetic molecule binding in the pore of an Na channel in a manner that promotes Na inactivation. The molecule can reach its binding site from the intracellular solution if activation and inactivation gates are both open. It can also reach the site from the membrane phase even if one or both of the gates are closed. The binding site has an important hydrophobic component (shading) and closure of the inactivation gate enhances the hydrophobic interaction [66].

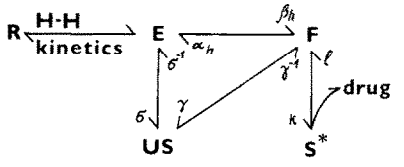


Fig. 10. Probable relationship between the ordinary (fast), drug-induced (slow) and ultraslow sodium inactivation in nerve and muscle membrane. Explanation in text.

cally resembles that caused by high external K^+ [63, 68] (compare Fig. 8A–D). In both cases (high K^+ and procaine) an increase of external concentration of Ca^{2+} shifts h_∞–E and s_∞–E curves in the direction of more positive E values and at the same time increases the level of s_f^{min}. These data could be easily explained if one assumes that the hypothetical R_2 receptor associated with slow inactivation is localized externally, and that Ca^{2+} ions compete with LA and K^+ for this receptor. It is worth to remind here that Ca^2_+ does not affect the decrease of $\bar{P}_{Na}$ caused by LA [62, 63].

For the problem in question, of special interest is the fact that in myelinated nerve procaine and trimecaine when applied internally decrease $\bar{P}_{Na}$ but do not induce a slow SI [63].

It is quite clear that some new experiments are required to elucidate the problem of a number and localization of the receptor for different LA in sodium channels.

In conclusion we would like to present in Fig. 10 the general kinetic scheme which describes the probable relationship between the ordinary (fast), drug-induced (slow) and ultra-slow SI in nerve and muscle membrane.

R, E, F, US and S* are the states of Na channels: resting, activated, fast inactivated, slow inactivated (ultra-slow), drug-induced slow inactivated, respectively. $\alpha_h, \beta_h, \sigma, \sigma^{-1}, \gamma, \gamma^{-1}, k, l$ are the forward (dominating during depolarization) and backward (dominating during repolarization) rate constants of the corresponding reactions.

The left part of the scheme concerned with the ultraslow inactivation has been proposed recently by Rudy [8, 69] and the right part ('drug-induced SI') was discussed above.

REFERENCES

1. A. Hodgkin and A. Huxley, *J. Physiol., Lond.* **117**, 500 (1952).
2. B. I. Khodorov, *The Problem of Excitability*. Plenum Press, New York (1974).
3. B. Hille, in *Handbook of Physiology*, Vol. I, Ch. 4, U.S.A. (1976).
4. I. Tasaki, *Nerve Excitation. A Macromolecular Approach*, Ch. Thomas, U.S.A. (1968).
5. C. Armstrong, F. Bezanilla and E. Rojas, *J. gen. Physiol.* **62**, 375 (1973).
6. E. Rojas and B. Rudy, *J. Physiol., Lond.* **262**, 501 (1976).
7. C. Sevčic and T. Narahashi, *J. Mem. Biol.* **24**, 329 (1975).
8. B. Rudy, Ph.D. Thesis, University of Cambridge (1976).
9. D. Eaton, G. Brodwick, G. Oxford and B. Rudy, *Nature, Lond.* **271**, 473 (1978).
9a. S. Oxford, C. Wu, and T. Narahashi, *J. gen. Physiol.* **71**, 227 (1978).
10. W. Chandler and H. Meves, *J. Physiol., Lond.* **211**, 707 (1970).
11. G. Oxford, I. Pooler and T. Narahashi, *J. Mem. Biol.* **36**, 159 (1977).
12. R. Stämpfli, *Experientia* **30**, 505 (1974).
13. F. Conti, B. Hille, B. Neumcke, W. Nonner and R. Stämpfli, *J. Physiol., Lond.* **262**, 729 (1976).
14. C. Bergman, I. Dubois, E. Rojas and W. Rathmayer, *Biochim. biophys. Acta* **455**, 173 (1976).
15. G. Romey, I. Abita, H. Schwaitz, G. Wunderer and M. Lazdunski, *Proc. natn. Acad. Sci. U.S.A.* **73**, 4055 (1976).
16. E. Koppenhöfer and H. Schmidt, *Pflügers Arch. ges. Physiol.* **303**, 153, 161 (1968).
17. T. Narahashi, B. Shapiro, T. Deguchi, M. Scuka and C. Wang, *Am. J. Physiol. 222*, 850 (1972).
18. G. Romey, E. Chicheportiche and M. Lazdunski, *Biochem. biophys. Res. Commun.* **64**, 1, 115 (1975).
19. Z. Krutetzkaja, Thesis, Leningrad University (1977).
20. G. N. Mozhayeva, A. P. Naumov, E. V. Grishin and N. M. Soldatov, *Biofisika* (in Russian) (1978, in press).
21. O. Schmitt and M. Schmidt, *Pflügers Arch. ges. Physiol.* **333**, 51 (1972).
22. W. Catterall, *J. biol. Chem.* **251**, 18, 5528 (1976).
22a. S. Singer, *J. Colloid. Interface Sci.* **58**, 3, 452 (1977).

23. J. Fox, *Biochim. biophys. Acta* **426**, 232 (1976).
24. W. Adelman and Y. Palti, *J. gen. physiol.* **54**, 589 (1969).
25. M. Meves and W. Vogel, *J. Physiol., Lond.* **267**, 395 (1977).
26. M. Cahalan, *J. Physiol., Lond.* **244**, 511 (1975).
27. R. Hoyt, *Biophys. J.* **3**, 399 (1965).
28. R. Hoyt, *Biophys. J.* **66**, 1074 (1968).
29. L. Goldman and C. Schauf, *J. gen. Physiol.* **59**, 659 (1972).
30. E. Peganov, *Bull. exp. Biol. Med. U.S.S.R.* **11**, 5, 1973.
31. F. Bezanilla and C. Armstrong, *J. gen. Physiol.* **70**, 549 (1977).
32. E. Albuquerque and J. Daly, in *Specificity and Action of Animal, Bacteria and Plant Toxins*. Ser. B (Ed. P. Caustercasas), p. 299. (1976).
33. B. I. Khodorov, E. M. Peganov, S. V. Revenko and L. D. Shishkova, *Brain Res.* **84**, 541 (1975).
34. B. I. Khodorov, E. M. Peganov, S. V. Revenko and L. D. Shishkova, *Proc. Int. Union Physiol. Sci.* **XII**, i95 (1977).
35. E. M. Peganov, B. I. Khodorov, S. V. Revenko and L. D. Shishkova, *Molec. Biol.* (in Russian), **16**, 42 (1976).
36. B. I. Khodorov, in *Ion Transport Processes* (Eds D. Tosteson, Yu. A. Ovchinnikov and R. Latorre) Vol. 2, p. 153. Raven Press, New York (1978).
37. S. V. Revenko and B. I. Khodorov, *Neurophysiology* (in Russian), **2**, 313 (1977).
38. A. Schmidt and O. Schmitt, *Pflügers Arch. ges. Physiol.* **49**, 133 (1974).
39. G. N. Mozhayeva, A. P. Naumov and Y. A. Negulajev, *Neurophysiology* (in Russian) **8**, 152 (1976).
40. G. N. Mozhayeva, A. P. Naumov, Y. A. Neguleyev and E. D. Nozyreva, *Biochim biophys. Acta* **466**, 461 (1977).
41. S. V. Revenko, *Neurophysiology* (in Russian) **9**, 544 (1977).
42. S. V. Revenko, Thesis (in Russian) Vishnevsky Surgery Institute (1977).
43. W. Catterall, *Proc. natn Acad. Sci. U.S.A.* **72**, 5, 1782 (1975).
44. W. Catterall, *J. biol. Chem.* **250**, 4053 (1975).
45. L. Huang, G. Ehrenstein and W. Catterall, *Biophys. J.* **17**, 268 (1977).
45a. J. Warnick, E. Albuquerque, R. Onur, S. Jansson, J. Daly, T. Tokuyama and B. Witkop, *J. Pharmac. exp. Ther.* **193**, 232 (1975).
46. W. Ulbricht, *A. Rev. Biophys. Bioengng* **6**, 7 (1977).
47. C. Armstrong and F. Bezanilla, *J. gen. Physiol.* **70**, 5, 567 (1978).
47a. B. Khodorov, *Neuroscience*, in press.
48. E. M. Peganov and B. I. Khodorov, *Bull. exp. Biol. Med. U.S.S.R.* **10**, 515 (1977).
49. F. Bezanilla and C. Armstrong, in *Cold Spring Harbor Symposium on Quant. Biol.* **XY**, 297 (1976).
50. H. Meves and W. Vogel, *J. Physiol., Lond.* **267**, 377 (1977).
51. C. Armstrong, *J. gen. Physiol.* **58**, 413 (1971).
52. C. Armstrong and B. Hille, *J. gen. Physiol.* **59**, 388 (1972).
53. G. Strichartz, *J. gen. Physiol.* **62**, 37 (1973).
54. B. Shapiro, *J. gen. Physiol.* **69**, 915 (1977).
55. J. Yeh and T. Narahashi, *J. gen. Physiol.* **69**, 293 (1977).
56. J. Yeh and T. Narahashi, *Fedn Proc.* **35**, N3 (1976).
57. C. Armstrong, *Q. Rev. Biophys.* **7**, 179 (1974).
58. M. Cahalan and B. Shapiro, *Biophys. J.* **16** (2, Pt 2), 76a (1976).
59. W. Almers and M. Cahalan, *Biophys. J.* **17**, 205 (1977).
59a. J. Yeh and C. Wu, *Biophys. J.* **21**, M-PM-DIO (1978).
59b. M. Cahalan, *Biophys. J.* **23**, 159 (1978).
60. N. V. Khromov-Borisov and M. J. Michelson, *Pharmac. Rev.* **18**, 3, 1051 (1966).
61. P. Årchem and B. Frankenhaeuser, *Acta physiol. scand.* **91**, 11 (1974).
62. B. I. Khodorov, L. D. Shishkova and E. M. Peganov, *Bull. exp. Biol. Med. U.S.S.R.* **3**, 13, (1974).
63. B. I. Khodorov, L. D. Shishkova, E. M. Peganov and S. V. Revenko, *Biochim. biophys. Acta* **433**, 409 (1976).
64. B. Hille, K. Courtney, and R. Dum, in *Molecular Mechanisms of Anesthesia* (Ed. B. Fink) *Progress in Anesthesiology* Vol. 1, p. 13. Raven Press, New York (1975).
65. B. Hille, *J. gen. Physiol.* **69**, 497 (1977).
66. B. Hille, *J. gen. Physiol.* **69**, 497 (1977).
67. W. Schwarz, P. Palade and B. Hille, *Biophys. J.* **20**, 343, 1977.
68. E. M. Peganov, B. I. Khodorov and L. D. Shishkova, *Bull. exp. Biol. Med. U.S.S.R.* **9**, 15 (1973).
69. B. Rudy, *Biophys. J.* **17**, 45a (1977).