Biochimica et Biophysica Acta, 433 (1976) 409–435
© Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

BBA 77270

INHIBITION OF SODIUM CURRENTS IN FROG RANVIER NODE TREATED WITH LOCAL ANESTHETICS

ROLE OF SLOW SODIUM INACTIVATION

B. KHODOROV, L. SHISHKOVA, E. PEGANOV and S. REVENKO

Vishnevski Surgery Institute, Moscow 113093 (U.S.S.R.)

(Received September 19th, 1975)

SUMMARY

Effects of different local anesthetics on sodium permeability were studied in single nerve fibres of frog by the method of voltage clamp. Inhibition of sodium currents by externally applied tertiary anesthetics, procaine and trimecaine, was the sum of a potentially independent block (reduced $\bar{P}_{Na}$) and slow sodium inactivation with time constants ranging from tens to hundreds of *ms* depending on membrane potential (at room temperature). Externally applied uncharged benzocaine produced a potentially independent block only. According to dose-response curves both processes are one-to-one reactions. In the case of trimecaine equilibrium constant the reaction responsible for reduction of $\bar{P}_{Na}$ is about 0.3 mM, while that for slow inactivation is more than ten times less (0.02 mM). Increasing pH from 5.6 to 8.5 markedly accelerated the slow inactivation process at all potential values. Divalent cations Ca^{2+} and Ni^{2+} shifted the steady-state slow inactivation curve along the potential axis and simultaneously reduced slow inactivation at the saturation level. Permanently charged quaternary trimecaine was ineffective when applied externally. Internally applied tertiary anesthetics and quaternary trimecaine as well as externally applied quaternary derivative of lidocaine QX-572 produced a progressively irreversible block enhanced by depolarization and inhibition reversibly increased by repetitive short-term depolarization (frequency-dependent inhibition). Inhibition of sodium currents by repetitive stimulation observed also in the case of externally applied tertiary anesthetics is due mainly to slow inactivation. The data suggests the existance of several types of receptor sites through which local anesthetics exert their blocking action on sodium permeability.

INTRODUCTION

Weidmann [1] was the first to suggest the involvement of sodium inactivation in the blocking action of local anesthetics on the excitable membrane. This idea was later developed by Khodorov and collaborators [2–5]. In experiments on the single Ranvier node the authors have systematically investigated the dependence of a

blocking effect of local anesthetics on the membrane potential, ionic composition and pH of the media. It has been shown that all agents which reduce resting sodium inactivation (hyperpolarizing current, divalent cations, low pH) weaken also the blocking potency of local anesthetics. However, this hypothesis met serious difficulty in explaining the fact that restoration of action potentials suppressed by anesthetics needs hyperpolarization over a period of hundreds of ms [3, 6], whereas the time constant of sodium inactivation τ_h is about 2–3 ms. It was therefore necessary to assume that either local anesthetics considerably slow down the removal of sodium inactivation or that it is due to some other as yet unknown slow process.

Studies of sodium currents by the method of voltage clamp in squid giant axons [7–9] and nerve fibres of the frog [10] revealed no essential changes in the time constant of sodium inactivation τ_h and $h_\infty - E$ relation under the action of local anesthetics. Therefore a conclusion was made that the inhibition of sodium current induced by these drugs may simply be described by reducing the sodium permeability constant, $\bar{P}_{Na}$.

However, it turned out later that sodium inactivation is a multicomponent process along with classical "fast", slow inactivation does exist [11] and contributes largely to the blocking action of procaine [12].

A comparative study of the mode of action of different local anesthetics-tertiary amines (procaine, trimecaine), benzocaine and quaternary amines (QT, QX-572) led us to the conclusion that several mechanisms underlying the blocking action of local anesthetics on the Ranvier node do exist: a decrease of $\bar{P}_{Na}$, slow sodium inactivation and "frequency-dependent" ("use-dependent") blockage of sodium channels, described by Strichartz [13].

The contribution of each mechanism to the total effect of a particular anesthetic depends on its structure, form (charged or neutral) mode of application to the membrane (external or intra-axonal) and membrane potential. Short reports on the data were published elsewhere [14, 15].

METHODS

The experiments were performed on single nerve fibres of the frog *Rana ridibunda* by the voltage clamp method of Dodge and Frankenhaeuser [16]. The schematic representation of nerve fibre preparation and voltage clamp circuit are shown in Fig. 1. To study internal action of anesthetics the internode in pool C was cut in an isosmotic KCl solution containing the test drug.

In several experiments, procaine and trimecaine (see ref. [17]) were administrated through the cut end in pool E, containing isosmotic KCl or CsF solutions. All experiments were done at room temperature (19–25 °C) with an external solution containing (in mM): NaCl 112, KCl 0, $CaCl_2$ 2. Potassium-free Ringer solution was used in order to eliminate slow sodium inactivation depending on outside potassium [11, 18]. 10 mM of tetraethylammonium chloride were added to block potassium currents. pH was made 7.3 unless otherwise stated. A buffer system glycylglycine-piperazine was used.

The structures of the local anesthetics used are shown in Fig. 2.

Available sodium permeability (P_{Na}) was measured in the usual way from peak sodium currents in response to short depolarizing test pulses to absolute mem-

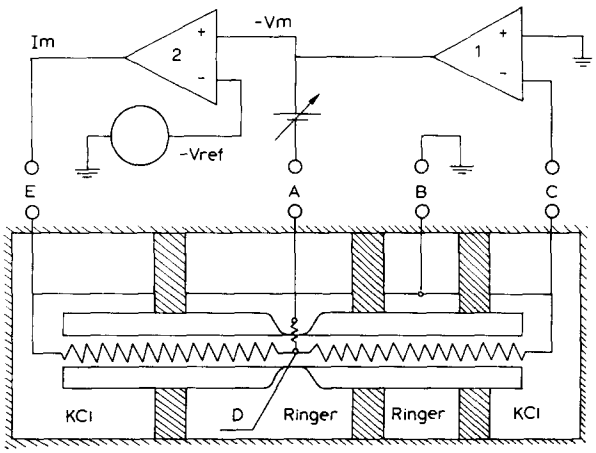

Fig. 1. The myelinated fibre preparation and voltage clamp circuit. A single fibre is mounted in lucite chamber with four pools. The node under investigation is in pool A. Vaseline seals are applied over the fibre at the partitions. Ringer solution is in pool B. Ringer plus test drug in pool A, fibre is cut at the internodes in isotonic KCl in pools C and E. Voltage clamp circuit is connected to fiber preparation through four calomel cells at the points C, B, A and E. Amplifier 1 operates to measure the membrane potential, amplifier 2 operates to clamp the membrane potential to a preselected value determined by pulse generator.

brane potentials between −20 and 0 mV. To eliminate initial sodium inactivation, both fast and slow, the membrane was held at high negative potentials through all the experiment (holding potential E_h between −95 and −110 mV). Moreover, in the experiments studying the effect of drug on $\bar{P}_{Na}$ a hyperpolarizing prepulse up to −125

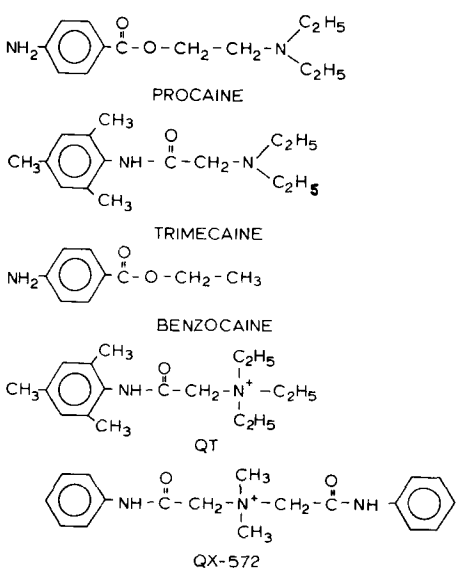

Fig. 2. Chemical structure of local anesthetics used.

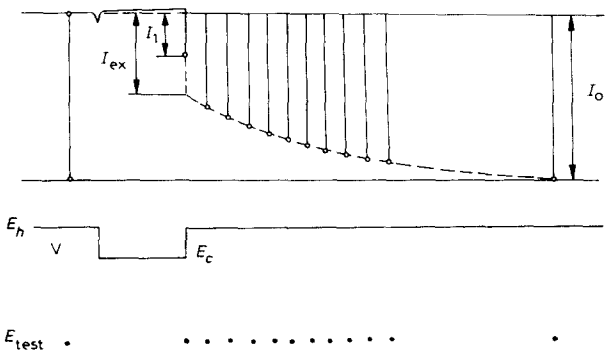

Fig. 3. Illustration of the procedure used to study slow sodium inactivation. Recovery of sodium currents inhibited by depolarizing conditioning prepulse (E_c) made from high negative E_h was traced by test pulses delayed to different time intervals. Slow component of the recovery curve was extrapolated to the end of conditioning step to obtain a quantitative measure (I_{ex}) of the relative number of sodium channels free from slow inactivation at that moment. Peak sodium current (I_1) during test pulse applied immediately at the end of E_c is a usual measure of sodium channels available for excitation (h).

mV for 1 s was applied before each test pulse. By a depolarizing conditioning prepulse of various amplitude and duration, dosed slow inactivation was brought about. The recovery from inactivation was traced by test pulses made with varying delay after the end of the prepulse. In some cases a series of short test pulses (1 ms long, pulse rate 10 per s) was used in order to shorten the time required to obtain steady state values and kinetic curves of the slow process for a number of membrane potential values. In such cases a correction was made on the interference of the "frequency-dependent inhibition" [13]. By extrapolating the slow component of the recovery curve of peak sodium currents to the moment at the end of the conditioning step we obtained a quantitative measure of the slow inactivation state at this moment (Fig. 3). As such a parameter, we used the ratio(s) of the extrapolated value of sodium current (I_{ex}) to the peak sodium current obtained without conditioning depolarization ($s = I_{ex}/I_0$, Fig. 3). The variable h of fast inactivation was measured simultaneously by applying a test pulse immediately at the end of the conditioning prepulse. By varying the amplitude and duration of the conditioning step studies were made of the voltage dependence of variables s and h and the time course of the slow process.

RESULTS

1. Effect of external procaine and trimecaine on the permeability constant $\bar{P}_{Na}$

In experiments on the Ranvier node, most researchers (except [19]) set the holding potential E_h at the level of the resting potential (−70–75 mV) and before each test pulse apply a hyperpolarizing prepulse of 40–50 ms duration. It is assumed that hyperpolarization of such a duration ensures complete elimination of the initial sodium inactivation since the time constant of the inactivation process τ_h at room temperature does not exceed 2–4 ms.

However Hodgkin-Huxley equations [20] do not take into account the fact

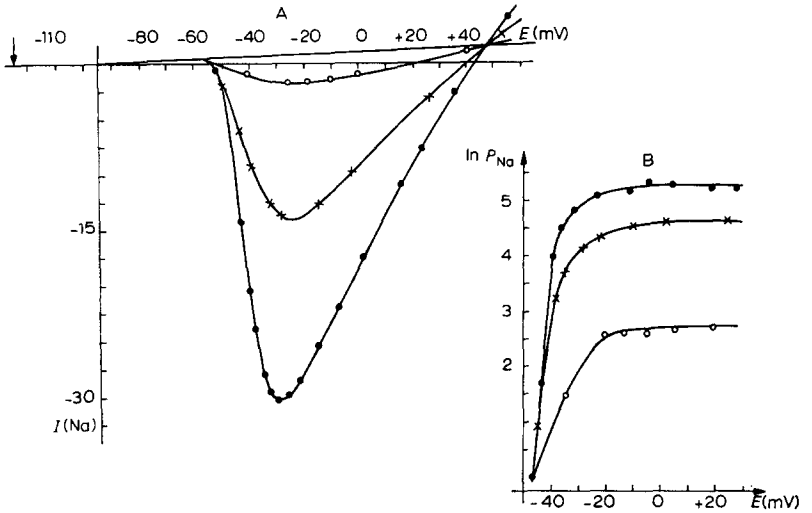

Fig. 4. Peak membrane currents (A) and sodium permeability, P_{Na}, (B) plotted as a function of membrane potential before (filled circles) and after external application of trimecaine in concentrations 0.036 mM (crosses) and 1.8 mM (open circles). Leakage current is shown by the straight line. Holding potential $E_h = -95$ mV, each test potential step was preceded by a 1 s long prepulse to -125 mV. Fibre 14.01.75.

that along with fast there is also a slow sodium inactivation with a time constant of about hundreds of milliseconds and more. To eliminate this inactivation it is necessary to hyperpolarize the membrane over a period of seconds. As long as the node is in the normal Ringer solution, the fraction of sodium channels which is in the state of slow inactivation at a resting potential is relatively small and in a number of cases it may be ignored. However, in potassium-rich external solution or under the action of anesthetics [12, 15] this fraction increases to such an extent that ignoring it leads to serious errors in the evaluation of $\bar{P}_{Na}$. It is precisely for this reason that we set E_h at the level of -95 to -110 mV.

Fig. 4A shows that the addition of trimecaine to the external solution led to a decrease in the peak sodium currents at all E values. P_{Na} values were calculated with the aid of the constant field equation [21] by the peak sodium currents Fig. 4B. The anesthetic substantially decreased saturation level of P_{Na} without affecting the position of the $P_{Na}-E$ curve with respect to the voltage axis. The reversal potential of sodium current remained unchanged so the decrease of sodium currents can be attributed to the decrease of the sodium permeability constant, $\bar{P}_{Na}$. Similar data were also obtained in other experiments with procaine and trimecaine. A shift of the permeability curve in the direction of positive E values was observed only in two experiments with procaine.

The measurements were plotted as a dose versus response curve in Fig. 5A. Here permeabilities are expressed as fractions of maximal peak P_{Na} obtained in potassium-free Ringer solution without any anesthetic. If it is assumed that one molecule of the drug binds to the receptor site R_1 to block one channel:

$$A + R_1 \underset{K_2}{\overset{K_1}{\rightleftarrows}} AR_1$$

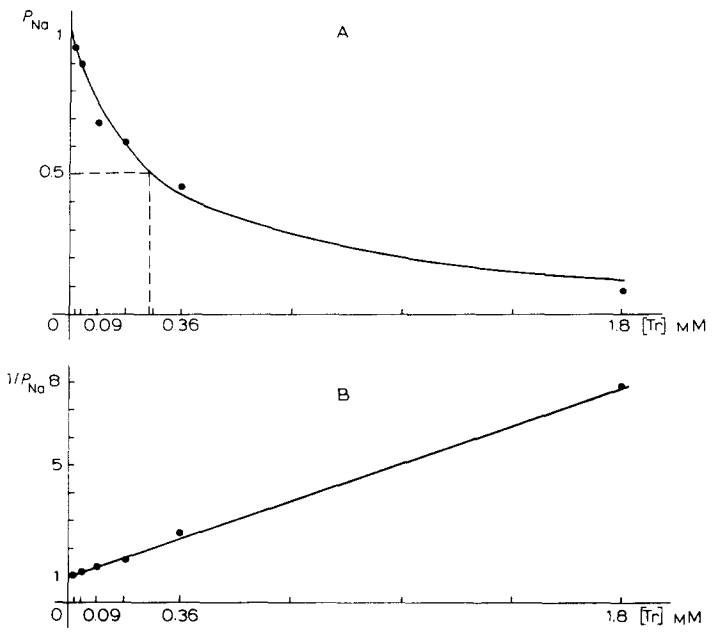

Fig. 5. Dependence of maximum value of peak sodium permeability on external concentration of trimecaine, [Tr]. Permeabilities are given in fractions of max peak P_{Na} obtained in Ringer solution. Dissociation constant for inhibitory drug binding is determined from the slope of the line in the plot $1/P_{Na}$ (B). Same fibre as in Fig. 4.

then $1/P_{Na}$ should be linearly related to the concentration of anesthetic [A]:

$$1/P_{Na} = 1 + [A]/K$$

where $K = K_2/K_1$ is a dissociation constant. Fig. 5B shows that this is actually the case. According to this plot the equilibrium constant for trimecaine is about 0.3 mM.

2. Slow sodium inactivation induced by procaine and trimecaine

Earlier [11] it was shown that slow inactivation does not develop in a potassium-free Ringer solution. In this case sodium currents recover from inactivation in several tens of milliseconds (with the time constant τ_h). If procaine or trimecaine is added to potassium-free outside solution then the restoration process following long enough depolarizing prepulse along with a fast component with almost unchanged time constant (τ_h) shows the slow component. This slow component of the recovery process has an exponential time course. Time constant (τ_s) of the slow recovery process at high negative E_h is independent of the amplitude of the conditioning prepulse as well as of the concentration of anesthetic but it is larger for procaine than for trimecaine (Table I and II). The time constant of the slow inactivation development under the depolarizing conditioning step is inversely related to the conditioning potential. As conditioned prepulse is made more positive τ_s decreases to a certain limiting value for a given concentration of drug (Table 1 and II). This limiting value decreases with increasing drug concentration (Table 1).

The curve of the steady state slow inactivation $s_\infty - E$ (Fig. 6B) is in its shape

TABLE I

DEPENDENCE OF THE TIME CONSTANT OF SLOW SODIUM INACTIVATION (τ_s) AND OF s_∞^{min} ON CONDITIONING POTENTIAL (E) AND CONCENTRATION OF TRIMECAINE

Fibre	Concentration (mM)	E_h (mV)	E (mV)	τ_s (ms)	s_∞^{min}	Temperature (°C)
8.10.74	0.036	−113	−113	380	0.34	19
25.09.74	0.036	−100	−100	400	0.26	20
15.10.74	0.036	−100	−100	220	0.39	24
			− 70	130		
			− 52	90		
23.09.74	0.036	− 90	− 90	400	0.40	—
24.09.74	0.036	− 80	− 80	410	0.23	—
3.12.73	0.072	− 95	− 95	320	0.32	—
28.11.73	0.072	−110	−110	150	0.29	—
17.06.74	0.09	− 95	− 95	250	0.20	23
16.10.74	0.09	−100	−100	200	0.23	25
			− 50	50		
9.07.74	0.09	− 95	− 95	200	0.16	25
30.01.75	0.09	− 95	− 95	290	0.20	23
			− 70	120		
			− 52	70		
			− 37	70		
14.06.74	0.09	−100	−100	180	0.30	—
25.09.74	0.18	−100	−100	370	0.10	20
15.10.74	0.18	−100	−100	220	0.25	24
			− 78	110		
			− 70	50		
			− 52	40		
15.09.74	0.18	−100	−100	330	0.04	—

and position along the voltage axis quite similar to that of fast inactivation $h_\infty - E$ (Fig. 6A, C). A striking difference is that slow inactivation is incomplete, i.e. s_∞ decreases to some non-zero level s_∞^{min}. The important point is that s_∞ reaches s_∞^{min} level just in the same potential region where h_∞ become close to zero (Figs. 6, 9–11). It should be emphasized that precisely in this potential region the time constant of the slow process also becomes independent of potential. The s_∞^{min} level decreases with increasing drug concentration. Fig. 7 shows s_∞^{min} values obtained for different trimecaine concentrations. As it can be seen the scattering of points is rather significant, so to obtain the best theoretical fit to experimental points the method of least squares was used. Curves 1 and 2 were calculated with the assumption that two (Curve 1) or one (Curve 2) molecule of anesthetic interact with the corresponding receptor (R_2) to inactivate one sodium channel. As can be seen Curve 1 greatly deviates from the experimental points for the low concentrations. According to Curve 2 ,which gives the best fit the equilibrium constant K of the reaction $A + R_2 \rightleftharpoons AR_2$ is 0.015 mM, i.e. it is more than ten-fold lower than that of the reaction involved in the reduction of sodium permeability parameter $\bar{P}_{Na}$.

Now let us look closer at the relations between the processes of inactivation described by the variables s and h. As we have mentioned above steady state slow inactivation reaches saturation in the region of potential values where steady state

TABLE II

DEPENDENCE OF THE TIME CONSTANT OF SLOW SODIUM INACTIVATION (τ_s) AND OF S_∞^{min} ON CONDITIONING POTENTIAL (E) AND CONCENTRATION OF PROCAINE

Fibre	Concentration (mM)	E_h (mV)	E (mV)	τ_s (ms)	S_∞^{min}
7.12.72	0.009	−110	−110	600	0.34
26.12.72	0.054	− 90	− 90	700	0.37
18.02.74	0.09	−100	−100	400	0.28
5.07.74	0.09	− 90	− 90	540	0.37
		− 90	− 42	130	
27.06.75	0.072	− 90	− 90	450	0.38
5.03.74	0.09	− 90	− 90	520	0.25
13.09.73	0.09	− 90	− 90	270	0.38
28.01.75	0.09	− 90	− 90	370	0.24
			− 42	180	
20.09.72	0.18	− 90	− 90	770	0.02
2.12.72	0.09	−120	−120	370	0.1
9.07.74	0.09	− 90	− 90	450	0.14
11.12.72	0.09	−100	−100	700	0.16
29.01.75	0.09	− 95	− 95	400	0.23
			− 68	340	
			− 52	250	
			− 37	250	
26.01.75	0.09	−100	−100	640	0.42
			− 48	240	
17.06.74	0.09	− 97	− 97	300	0.19

fast inactivation becomes complete. This holds true under various environmental conditions. The hypothesis therefore seems quite credible that slow inactivation is coupled to the fast one in such a way that only previously inactivated sodium channels can be transfered to the slow inactive state.

The relations between the fast and slow inactivation processes may be presented in the terms of chemical kinetics as follows:

$$\mathrm{H} \underset{\alpha_h}{\overset{\beta_h}{\rightleftharpoons}} \mathrm{F} \underset{\alpha_s}{\overset{\beta_s}{\rightleftharpoons}} \Sigma$$

where: H = either active or resting states of the sodium channel; F = fast inactive state; Σ = slow inactive state; α_h, β_h, α_s, β_s are the rate constants of the corresponding transitions indicated by the arrows.

Let h, f and δ be the fractions of the total number of sodium channels being in the states H, F and Σ respectively, and s be the fraction of the channels which are free from slow inactivation. Then we have the following relations:

$$h+f+\delta = 1;\ s = 1-\delta \tag{1}$$

$$\dot{h} = \alpha_h s-(\alpha_h+\beta_h)h \tag{2}$$

$$\dot{s} = \beta_s h+\alpha_s-(\alpha_s+\beta_s)s \tag{3}$$

$$h_\infty = \frac{\alpha_h}{\alpha_h+\beta_h} s_\infty \tag{4}$$

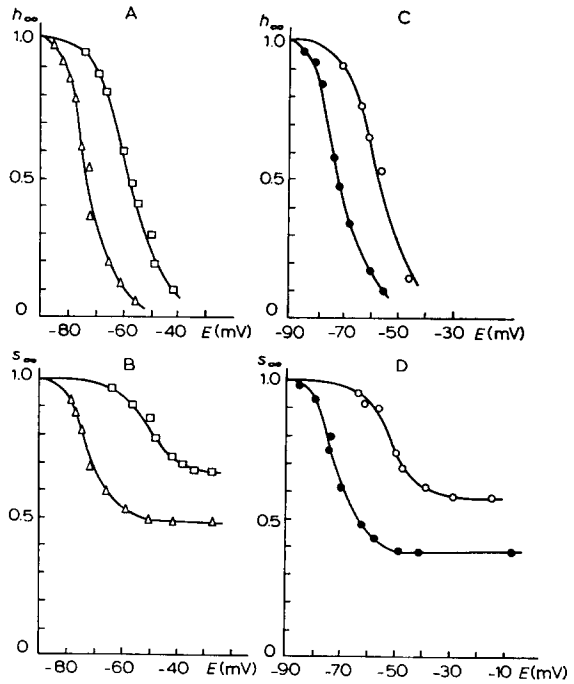

Fig. 6. Steady-state fast (h_∞) and slow (s_∞) sodium inactivation related to membrane potential at normal and increased outside calcium concentration. A, B: node in Ringer solution with 10 mM KCl (triangles) and 10 mM KCl plus 20 mM Ca^{2+} (squares). Fibre 19.03.73. C, D: node in Ringer solution containing 0.054 mM of procaine (filled circles) and 0.054 mM of procaine plus 20 mM Ca^{2+} (open circles) Fibre 26.12.72.

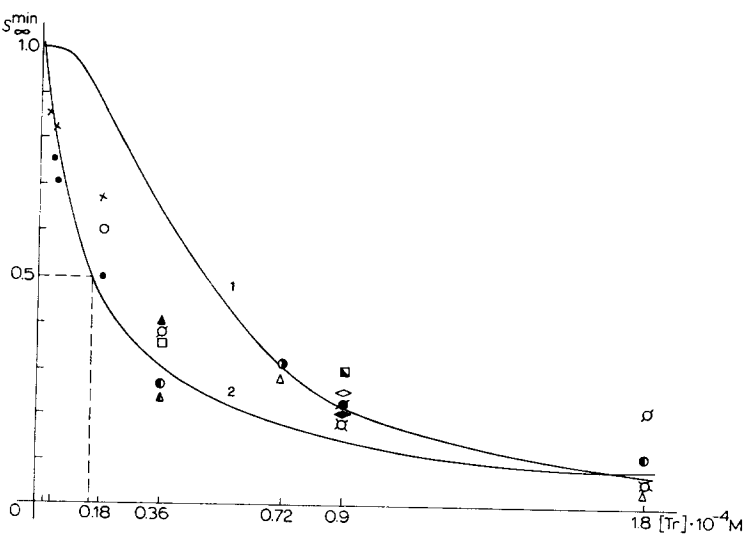

Fig. 7. Saturation level of slow inactivation ($s_\infty{}^{min}$) as a function of trimecaine concentration. Different figures refer to different fibres. Smooth lines are least squares curves computed according to the equation $S_\infty{}^{min} = (1+[Tr]^n/K)$, $n = 1$ for curve 2 and $n = 2$ for curve 1.

$$s_\infty = \frac{\alpha_s}{\alpha_s+\beta_s} + \frac{\beta_s}{\alpha_s+\beta_s} h_\infty \tag{5}$$

For high depolarizing potentials ($h_\infty = 0$):

$$s_\infty = s_\infty^{\min} \frac{\alpha_s}{\alpha_s+\beta_s} \tag{5a}$$

Considering that α_h and β_h rate constants are much larger then α_s and β_s we get for the time constant of slow process:

$$\tau_s = 1/(\alpha_s+\beta_s(1-h_\infty^*)) \tag{6}$$

where

$$h_\infty^* = \frac{\alpha_h}{\alpha_h+\beta_h} \tag{7}$$

Note that because of h_∞^* in Eqn. 6 τ_s will depend on membrane potential even if α_s and β_s are potentially independent.

At high depolarizations $h_\infty^* \to 0$ and

$$\tau_s = \tau_s^+ \approx 1/(\alpha_s+\beta_s) \tag{8}$$

For the rate constants we have:

$$\alpha_s^+ \approx s_\infty^{\min}/\tau_s^+ \tag{9}$$

$$\beta_s^+ \approx 1-s_\infty^{\min}/\tau_s^+ \tag{10}$$

On the contrary at high hyperpolarizing potentials, where $h_\infty^* \to 1$

$$\tau_s = \tau_s^- \approx 1/\alpha_s^- \tag{11}$$

The data available allows us to make some physical interpretations of the rate constants α_s and β_s. With an increase of the concentration of anesthetic τ_s^+ decreases while τ_s^- does not change (Table I, fibre 15.10.74). It follows that according to Eqns. 8 and 11, α_s does not change and β_s increases with increasing drug concentration. In the absence of anesthetic in potassium-free Ringer solution slow inactivation does not develop, i.e. $\beta_s = 0$.

So one can assume that the transition of sodium channels from the F to Σ state is a direct result of the drug molecule binding to the receptor which become accessible during depolarization in the inactivated channels. Then β_s is the rate constant of the binding and α_s is the rate constant of unbinding reactions. According to the dose-response curve for $s_\infty^{\min}$ (see above) one molecule of drug blocks one sodium channel, i.e. $\beta_s = \gamma\ [A]^1$. The data (Table 1, fibre 15.10.74) allows us to verify this proposition by using the kinetic parameter τ_s and the Eqns. 6, 8 and 11. In this case an assumption is made about the independence of α_s and β_s rate constants on the potential. The given experiment shows that the five-fold increase of the trimecaine concentration (from 0.036 to 0.18 mM) caused about 3.2-fold increase in β_s which corresponds to $\beta_s = \gamma[A]^{0.7}$. The calculation of $s_\infty^{\min}$ on the assumption that one molecule of anesthetic binds two R_2 receptors shows that $s_\alpha^{\min} = 0$ already

at 0.07 mM of trimecaine which is not the case (Fig. 6). Thus we come to the conclusion that one drug molecule is needed to block one channel. On the other hand this result may be interpreted (considering the independent verification of the one-

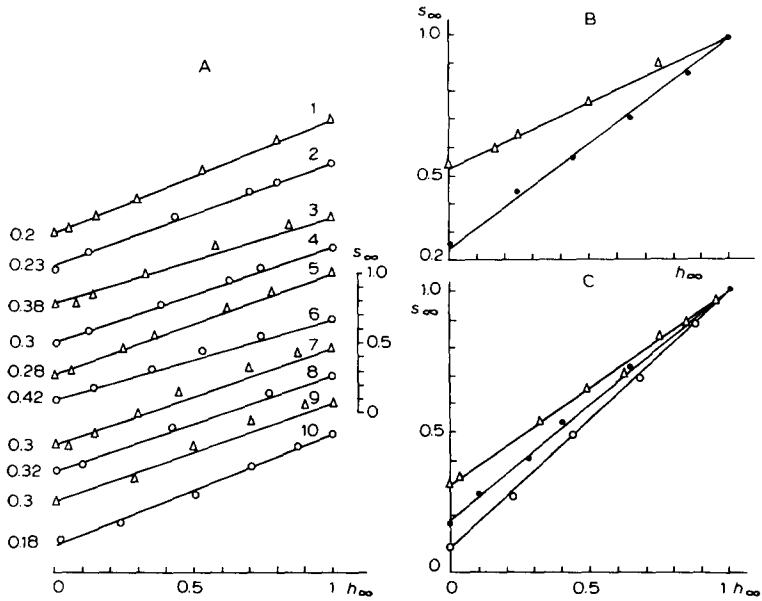

Fig. 8. Steady-state slow inactivation (s_∞) as a function of steady-state fast inactivation (h_∞) in the nodes treated with trimecaine and procaine. A: numbers 1–10 refer to different fibres. Curves 1 and 2 were obtained for 0.09 mM of trimecaine, curves 3–10 for 0.09 mM of procaine. Straight lines were drawn by eye. Numbers at the left at each line indicate $s_\infty{}^{min}$ level. B: node in Ringer solution containing 0.09 mM of procaine (filled circles) and after addition of 20 mM of Ca^{2+} (triangles). Fibre 7.12.72. C: filled circles; 0.09 mM of trimecaine, pH 7.3; triangles, trimecaine plus 20 mM of Ca^{2+}, pH 7.3; open circles, trimecaine, pH 6. Fibre 9.07.74.

TABLE III

RATE CONSTANTS OF THE SLOW INACTIVATION AT DIFFERENT CONDITIONED POTENTIALS (E)

Fibre	Drug (mM)	pH	E_h (mV)	E (mV)	α_s^- (10^{-3} ms^{-1})	E (mV)	α_s^+ (10^{-3} ms^{-1})
5.07.74	Procaine 0.09	7.3	− 90	− 90	1.8	−42	2.1
26.01.75	Procaine 0.09	7.15	−100	−100	1.5	−48	1.7
		8.5	−100	−100	2.5	−48	4.5
28.01.75	Procaine 0.09	7.3	− 90	− 90	2.7	−42	1.3
29.01.75	Procaine 0.09	7.3	− 95	− 95	2.4	−37	1.15
		8.5	− 95	− 95	5.0	−37	7.6
	+40 mM Ca^{2+}	7.3	− 95	− 95	3.0	−32	3.1
15.10.74	Trimecaine 0.036	7.3	−100	−100	4.4	−52	4.3
	Trimecaine 0.18	7.3	−100	−100	4.5	−52	6.0
	+20 mM Ca^{2+}	7.3	−100	−100	5.2	−48	11.0
16.10.74	Trimecaine 0.09	7.3	−100	−100	5.0	−50	4.6
30.01.75	Trimecaine 0.09	7.3	− 95	− 95	3.5	−37	5.0
		5.6	− 95	− 95	1.4	−37	0.35

to-one reaction assumption by the dose-response curve) as the evidence in favor of the assumption of potential independency of α_s and β_s rate constants.

A linear relation between h_∞ and s_∞ would testify in favor of the proposed mechanism with voltage independent rate constants of the slow process. In fact nearly linear relation was found in most of the cases (Fig. 8) as yet collected.

If, however such a dependence does exist it should be much less pronounced than that for α_h and β_h rate constants. The calculations of α_s according to Eqns. 9 and 11 by the data of several experiments with procaine and trimecaine do not reveal systematic differences between α_s at high depolarizations (α_s^+) and at high hyperpolarizations (α_{s-}) (Table III).

3. Influence of divalent cations on the effects of externally applied procaine and trimecaine

As follows from Figs. 6 and 9 and Table IV an increase of external concentration of Ca^{2+} or the addition of Ni^{2+} to the solution shifted $P_{Na}-E$, $s_\infty-E$ and $h_\infty-E$ curves in the direction of positive E values and at the same time increased the s_∞^{min} level in both procaine and trimecaine treated nodes. These are exactly the same effects which divalent cations produce in the case of potassium-induced slow

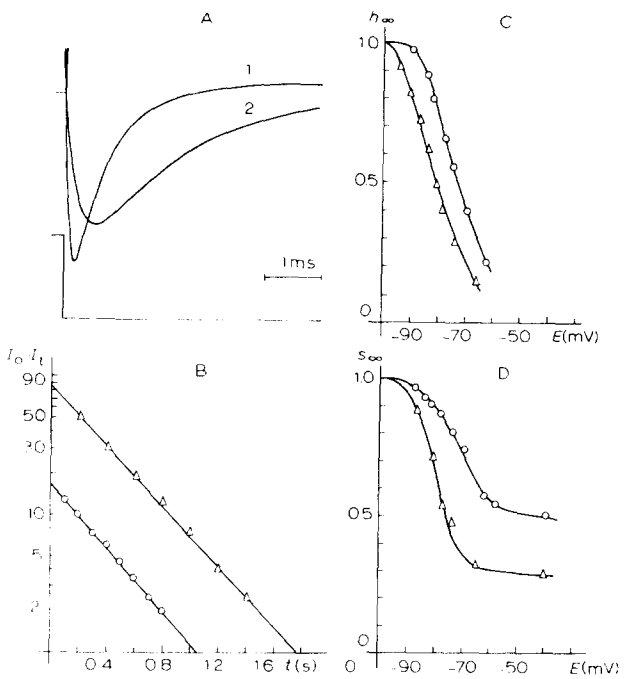

Fig. 9. Effects of Ni^{2+} on sodium currents in the node treated with procaine. A: records of sodium currents obtained in solutions containing 0.09 mM of procaine (1) and 0.09 mM of procaine plus 1 mM Ni^{2+} (2). B: semilogarithmic plot of the time course of restoration of sodium currents suppressed by a 1 s long depolarizing prepulse. C, D: steady-state fast (h_∞) and slow (s_∞) sodium inactivation. Throughout the figure triangles refer to the solution containing 0.09 mM of procaine; circles, same solution with 1 mM of Ni^{2+} added. Fibre 18.02.74.

TABLE IV

EFFECT OF DIVALENT CATIONS ON THE SLOW SODIUM INACTIVATION CAUSED BY PROCAINE AND TRIMECAINE

Fibre	Drug (mM)	Cation	E_h (mV)	E (mV)	τ_s (ms)	S_∞^{min}
18.02.74	Procaine 0.09	—	−100	−100	400	0.28
		1 mM Ni^{2+}	−100	−100	370	0.52
26.12.71	Procaine 0.09	—	− 90	− 90	700	0.37
		20 mM Ca^{2+}	− 90	− 90	620	0.57
7.12.72	Procaine 0.09	—	−110	−110	600	0.34
		40 mM Ca^{2+}	−110	−110	530	0.55
29.01.75	Procaine 0.09	—	− 95	− 95	410	0.34
			− 95	− 33	200	0.34
		40 mM Ca^{2+}	− 95	− 95	350	0.56
			− 95	− 32	180	
17.06.74	Procaine 0.09	—	− 95	− 95	250	0.20
		20 mM Ca^{2+}	− 95	− 95	250	0.43
15.01.74	Trimecaine 0.18	—	−100	−100	220	0.25
		—	−100	− 52	40	
		20 mM Ca^{2+}	−100	−100	190	0.44
			−100	− 48	40	
9.07.74	Trimecaine 0.09	—	− 95	− 95	220	0.16
		20 mM Ca^{2+}	− 95	− 95	210	0.34

inactivation (Fig. 6AB). Time constants τ_s^+ and τ_s^- of the slow process hardly changed under the influence of Ca^{2+} (Table IV). Only a slight tendency toward a decrease in τ_s^- may be noted. It is well known that Ni^{2+} sharply slow down the processes of activation and fast sodium inactivation [22]. It was also the case in the experiment of Fig. 8A. However Ni^{2+} failed to change the time course of the slow process (Fig. 9B).

Sodium permeability constant $\bar{P}_{Na}$ was not affected by Ca^{2+} [12]. This is in agreement with findings of Arhem and Frankenhaeuser [19]. The reason for the discrepancy of this result with the data of Blaustein and Goldman [23] who reported the enhancement of blocking action of local anesthetics by reducing $[Ca]_0$ may be due to the fact that the holding potential in these experiments was not negative enough with the consequence that they observed the influence of Ca^{2+} on slow inactivation.

The shift of the permeability curve and both slow and fast inactivation curves in the direction of positive E values can be easily explained by neutralizing fixed negative charges present on the membrane surface by divalent cations [24]. The qualitative account of the increase in the s_∞^{min} level does not also cause any special difficulty. It is well known that Ca^+ can compete with local anesthetics for certain chemical groups e.g. phosphate groups of phospholipids in the cell membrane [25–27]. The increase of s_∞^{min} in a calcium-rich solution is therefore tempting to explain by the binding of Ca^{2+} to receptor R_2.

5. Influence of pH on the effects of procaine and trimecaine

As tertiary amines procaine and trimecaine are present in the saline solution in protonated BH^+ and neutral B forms. The concentration ratio of the charged

form to the uncharged form can be calculated from the Henderson-Hassebalch equation:

$$\log \frac{BH^+}{B} = -pH + pK_a$$

To answer the question of which of these forms is active many researchers studied the influence of pH on the effects of anesthetics (see [28, 29]).

In our experiments a decrease of pH from 7.3 to 6.0 did not noticeably affected $\bar{P}_{Na}$ under the action of both procaine ($pK_a \sim 9.0$) and trimecaine ($pK_a \sim 7.9$) [17]. Only a shift in the permeability curve in the positive direction occured which is characteristic of the decrease in pH in the intact fibres (Fig. 10). Curves $h_\infty - E$ and $s_\infty - E$ also shifted in the same direction. In this respect the effect of the decreasing pH was similar to that of the increase of $[Ca]_0$ and apparently was due to the same factor, namely the neutralizing of negative surface charges. However, while $s_\infty{}^{min}$ always significantly increased under the influence of Ca^+ the decrease of pH reduced $s_\infty{}^{min}$ or did not affect it at all (Fig. 10, Table V).

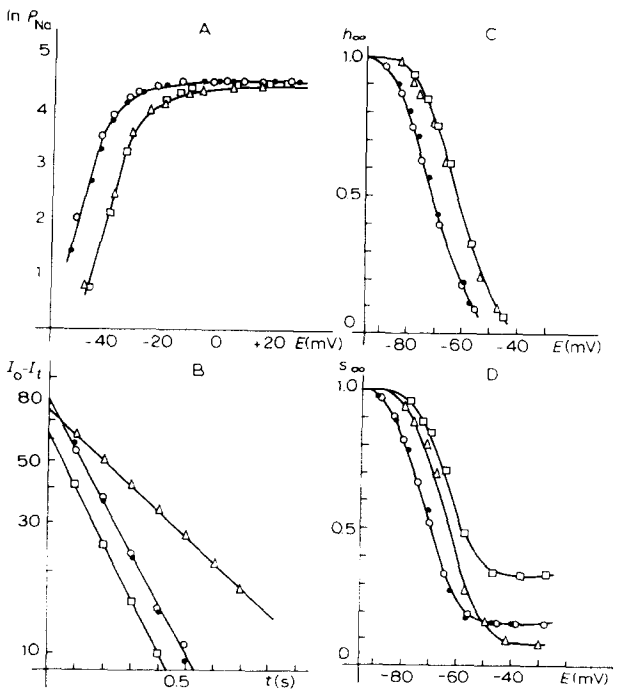

Fig. 10. Effects of the decrease of pH of external solution on sodium permeability (A), time course of slow inactivation (B) and steady-state fast (C) and slow (D) inactivation in the membrane treated with trimecaine. Experimental points were taken in the following way: open circles, node in Ringer solution containing trimecaine (0.09 mM), pH 7.3; triangles, pH of the above solution changed from 7.3. to 6.0; filled circles, pH brought back to 7.3; squares, outside calcium concentration increased to 22 mM, pH 7.3. In B semilogarithmic plots of the recovery of sodium currents from slow inactivation are presented. I_0 is the peak of I_{Na} under the test potential step made before conditioning depolarization; I_t, the peak of I_{Na} under the same test pulse applied at the moment t after the cessation of conditioning depolarization. Fibre 9.07.74.

TABLE V

EFFECTS OF pH CHANGES ON THE SLOW SODIUM INACTIVATION CAUSED BY PROCAINE AND TRIMECAINE

Fibre	Drug (mM)	pH	E_h (mV)	E (mV)	τ_s (ms)	s_∞^{min}
9.07.75	Trimecaine 0.09	7.3	− 95	− 95	200	0.16
		6.0	− 95	− 95	480	0.08
29.01.75	Procaine 0.09	7.3	− 95	− 95	400	0.23
				− 68	340	
				− 52	250	
				− 37	250	
		8.5	− 95	− 95	200	0.46
				− 69	85	
				− 53	60	
				− 37	60	
26.01.75	Procaine 0.09	7.15	−100	−100	640	0.42
				− 48	240	
		8.5	−100	−100	400	0.27
				− 48	60	
17.06.74	Procaine 0.09	7.3	− 97	− 97	300	0.19
		5.9	− 97	− 97	650	0.21
30.01.75	Trimecaine 0.09	7.3	− 95	− 95	290	0.20
				− 70	140	
				− 53	70	
				− 37	70	
		5.6	− 95	− 95	700	0.14
				− 70	540	
				− 53	400	
				− 37	400	

The increase of pH from 7.3 to 8.5 shifted the permeability curve in the direction of negative E values and magnified the effects of anesthetics on $\bar{P}_{Na}$ (Fig. 11). Steady state fast and slow inactivation curves were also shifted in the same direction. However the s_∞^{min} level in some cases considerably increased and in other decreased (Fig. 11, Table V). Whereas shifts in pH did not result in simple changes in s_∞^{min} level the time constants of slow process changed in a regular manner. Figs. 10 and 11 and Table V show that in all the experiments the decrease in pH caused a considerable increase in both τ_s^+ and τ_s^- while the increase in pH produced an opposite effect. In this respect the pH effects radically differ from those of Ca^{2+}.

One may suppose that hydrogen ions exert some specific inhibiting action on the reaction underlying the transition of sodium channels into the slow inactive state. However it was found that decreasing pH in potassium-rich solution (Fig. 12) produced no effect on the τ_s^+ and τ_s^-. In this case pH effects did not differ from those of outside calcium (Fig. 6). From these data it follows that the strong dependence of slow inactivation kinetics on pH in the case of local anesthetics is connected either with the pH influence on the $[BH^+]/[B]$ ratio or with some specific influence of hydrogen ions on the drug-to-receptor binding process or with both. We also examined a number of models of pH effects, in particular: (1) the model in which form B can interact with protonated receptor only and (2) a model which assumes two simultaneous reactions:

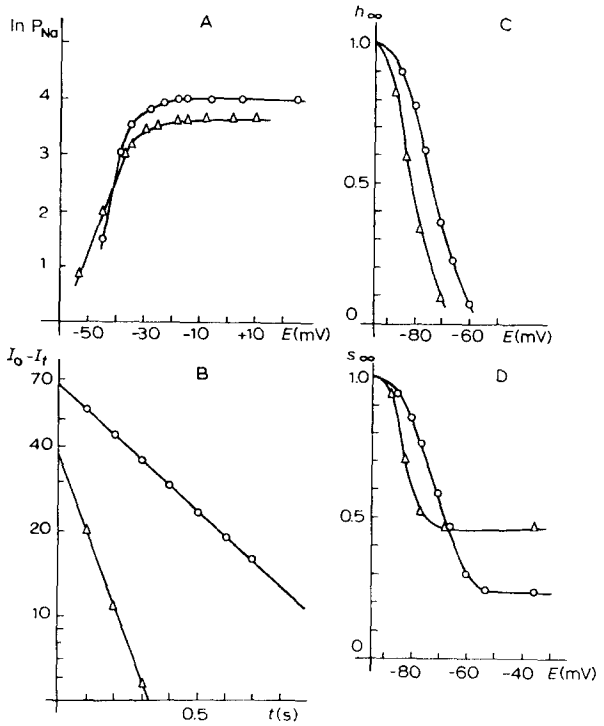

Fig. 11. Effects of the increase of pH on sodium permeability and inactivation in procaine treated node. External solution with pH 7.15 (circles) and 8.5 (triangles) contained 0.09 mM of procaine. A: logarithm of P_{Na} related to membrane potential. B: semilogarithmic plot of the time course of the recovery of sodium currents after a 1 s long depolarizing prepulse. C, D: steady-state fast (h_∞) and slow (s_∞) sodium inactivation. Fibre 29.01.75.

$$B + HR_2 \rightleftharpoons BHR_2$$

$$BH + R_2 \rightleftharpoons BHR_2$$

However neither model made it possible to fit the dependence of time constants of slow process on pH. From purely formal considerations it follows that the rate constants of both on (β_s) and off (α_s) reactions should increase upon increasing pH (since both τ_s^+ and τ_s^- are decreased). Since steady-state slow inactivation is a function of β_s: α_s ratio, $s_\infty^{min} = \alpha_s/\alpha_s + \beta_s$ will increase, decrease or remain unchanged depending on the relative change of β_s and α_s rate constants.

6. *Effects of external application of benzocaine and quaternary anesthetics QT and QX-572*

Unlike procaine and trimecaine, benzocaine does not have nitrogen in the alkyl chain and therefore hardly dissociates in neutral saline solutions. Nevertheless in a concentration of 0.3 mM benzocaine completely blocks the action potentials in the Ranvier node [30]. Our experiments have shown that this effect is due to the decrease in $\bar{P}_{Na}$ (Fig. 13A, B). Slow sodium inactivation does not develop under the action of benzocaine (Fig. 13C). Judging by the dose-response curve (Fig. 13A, B)

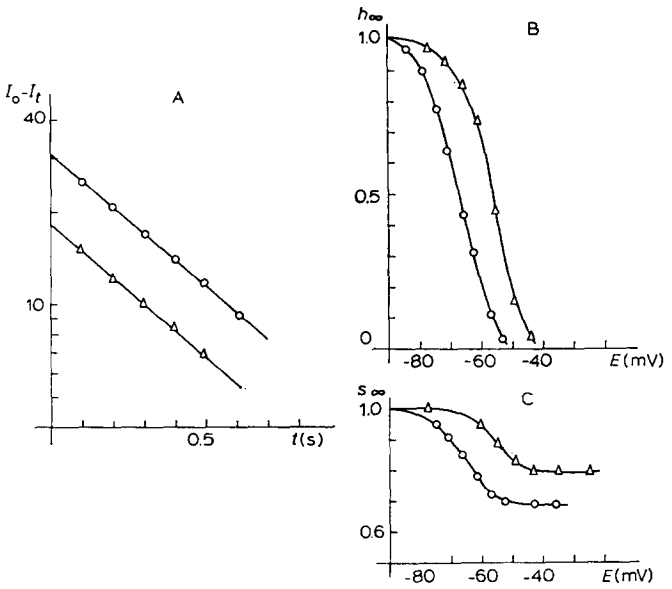

Fig. 12. The effects of pH on sodium inactivation in potassium rich external medium. External solution contained 10 mM KCl. Circles, pH 7.0; triangles, pH 6.0. A: time course of sodium currents recovery after a 1 s long depolarizing step (semilogarithmic plot). B, C: steady-state fast (h_∞) and slow (s_∞) inactivation. Fibre 30.07.74.

binding of benzocaine to the receptor is a one-to-one reaction. The dissociation constant of this reaction K was estimated as 0.4 mM which is very close to that for trimecaine and also to that obtained recently for benzocaine [19] on the *Xenopus laevis* fibres (0.49 ± 0.24).

Like QX-314 [13] quaternary derivative of trimecaine QT is ineffective when applied to the node from outside. In concentration 0.5 mM it caused no changes in the current voltage relation (Fig. 14A) and did not produce slow inactivation (Fig. 14B). Ineffectiveness of QT and QX-314 is, apparently, connected with its inability to penetrate into the lipid layers of the membrane. In favor of such an assumption is the fact that the quaternary derivative of lidocaine QX-572, which has two aromatic rings that make it lipid soluble, is capable of decreasing sodium permeability not only on intra-axonal, but also on external application [29, 31]. The decrease of sodium currents upon application of QX-572 proceeds slowly and reaches equilibrium only in 2–5 min. Depolarizing pulses cause a new steady decrease of sodium currents (Fig. 15). No signs of the restoration process which is characteristic of the slow inactivation was detected after a 1 s depolarizing step (Fig. 15B). Thus neither neutral benzocaine nor permanently charged QT and QX-572 applied from outside were capable of inducing slow sodium inactivation. The difference in the time course of development of the effect and the fact that only in the case of QX-572 depolarizing pulses enhance the action of drug on $\bar{P}_{Na}$ apparently indicates the different modes of action of QX-572 and such drugs as benzocaine, procaine and trimecaine.

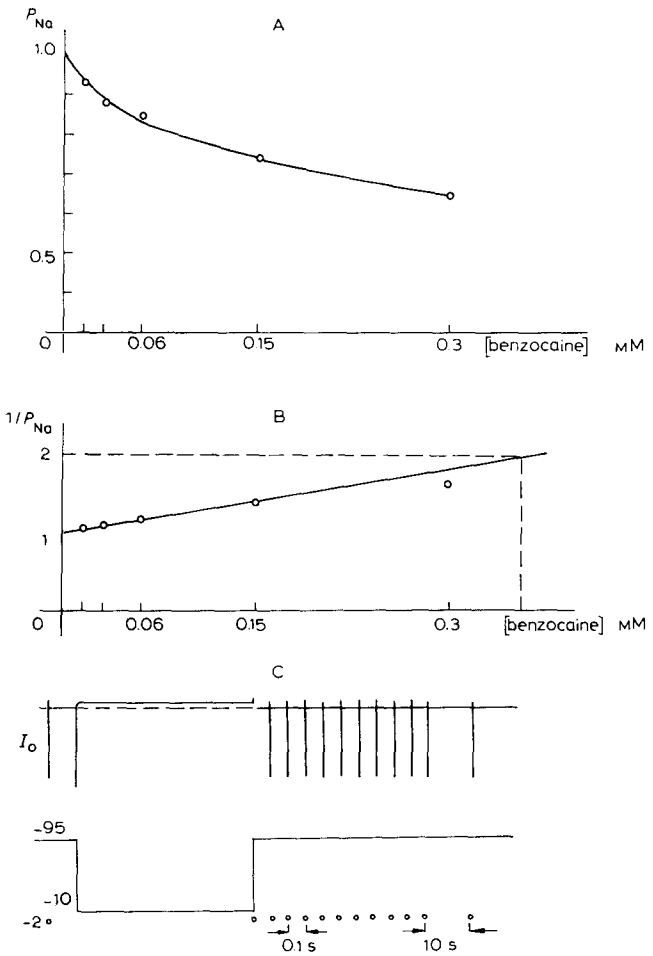

Fig. 13. The effects of benzocaine. A, B: sodium permeability and its reversed value related to external benzocaine concentration. C: sodium currents after a 1 s long conditioning prepulse. Fibre 24.03.75.

7. Intra-axonal administration of local anesthetics

The effects of intra-axonal application of QT were (Fig. 14C) very much similar to those observed under the externally applied QX-572. As anesthetic reached the node a decrease of sodium currents occured. In 30–40 min, (i.e. after the inhibition reached equilibrium), application of several prolonged (1 s) depolarizing pulses caused a further steady decrease of sodium currents. As in the case of QX-572 no recovery process indicative of slow inactivation was observed after a 1 s long depolarizing prepulse (Fig. 14D). A very similar picture was also observed during the intra-axonal application of tertiary amines procaine and trimecaine. But to obtain a visible effect on sodium permeability fairly high concentrations of drugs (about 4 mM) were needed to be applied to the out internode. Note that in the squid giant axon procaine is about equally effective on external and intra-axonal application [9]. The need in such high concentration for myelinated fibres may be due to high

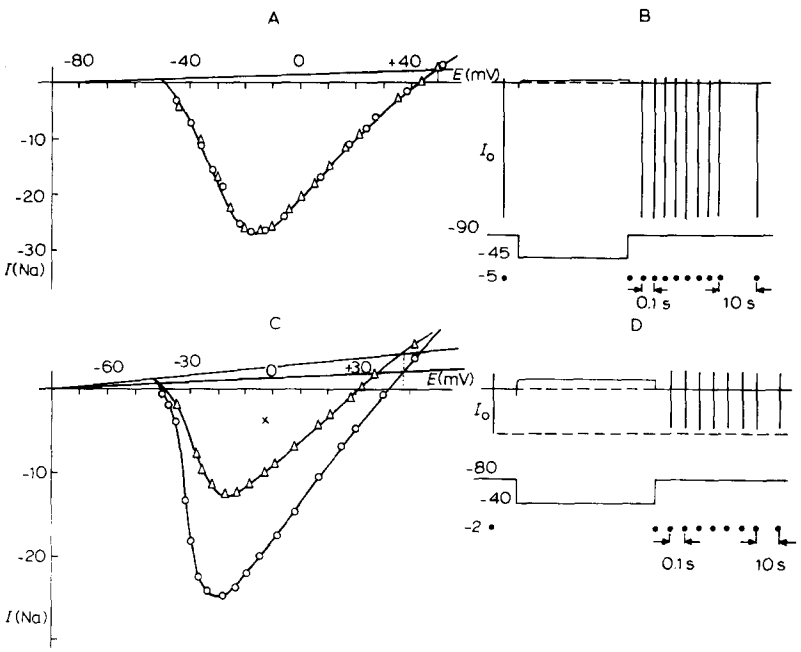

Fig. 14. Effects of quaternary trimecaine, QT. A: current-voltage relations taken before (circles) and during (triangles) external application of QT (0.5 mM). B: sodium currents after a 1 s long depolarizing step under the action of externally applied QT (0.5 mM). C: current-voltage relations taken 15 (circles) and 40 (triangles) min after cutting the internode in an isotonic KCl solution containing QT (0.5 mM); cross indicates the amplitude of inward current after several 1 s long depolarizing pulses. D: sodium currents before (I_o) and after a 1 s long depolarizing step (to −40 mV). Test steps to −2 mV are indicated by points. Fibre 5.11.73.

lipid-solubility of tertiary amine anesthetics which might make the myelin sheath a reservoir "pumping" the drug out of axoplasm.

And again, depolarizing pulses produced a considerable steady inhibition of sodium currents (Fig. 16A). Some recovery of sodium currents could be detected after the end of depolarization (Fig. 16B). However, the time course of this recovery was much slower (time constant of the order of several s) compared with that of the recovery from slow inactivation observed under the action of externally applied tertiary anesthetics (hundreds of ms).

8. Inhibition by repetitive stimulation

Strichartz [13] showed that the inhibition of sodium currents by internally applied quaternary derivatives of lidocaine QX-314 and QX-222 occured as a sum of a constant "tonic" phase and a reversible "phasic" inhibition accompanying short depolarizing pulses. This latter "use-dependent" (or "frequency dependent") inhibition of sodium currents accumulates in the series of impulses, inhibition depending on the pulse rate and accumulation taking place even at 1 s intervals between stimuli (temperature 6–7 °C). Steady, tonic inhibition was also enhanced by depolarization. On the basis of his findings Strichartz [13] suggested the existence of two kinds of receptors in the sodium channels. The first one (we shall call it R_3) responsible for

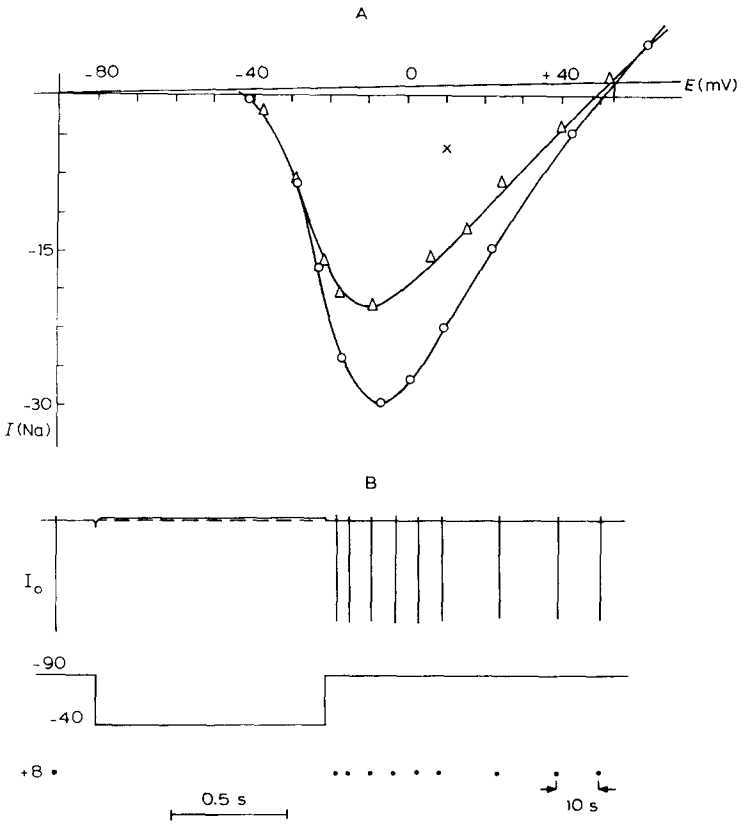

Fig. 15. Effects of quaternary derivative of lidocaine QX-572. A: current-voltage relations before (open circles) and 12 min after addition of QX-572 (0.136 mM) to the external solution; cross indicates peak sodium current after several 1 s long depolarizing pulses. B: sodium currents after a 1 s long depolarizing step (to −40 mV). Test pulses to +8 mV are indicated by dots. Leakage current is not substracted. Fibre 21.01.75.

the tonic inhibition located at the internal opening of the channel, is easily accessible and has very high affinity for QX. The binding of QX to this receptor site has a requirement that m^3 gates be open. The second receptor site (R_4) is located at some distance into the channel and becomes accessible from the axoplasmic side of the membrane when the channel is open. This binding site has a much lower affinity for QX. Later Hille et al. [29] and Courtney [31] found that use-dependent inhibition is also observed upon the external application of lidocaine (0.5 mM), its tertiary derivative GEA-968 (0.6 mM) and quaternary anesthetic QX-572 (0.5 mM). Hille et al. regard this fact as important evidence in favor of the hypothesis of the common mode of action of local anesthetics, both tertiary and quaternary amines. According to this hypothesis tertiary anesthetics in the form of neutral base diffuse across the membrane, become protonated in the axoplasm and in cationic form block the sodium channel binding to sites R_3 and R_4.

However, since only tertiary amine anesthetics were capable of producing slow inactivation the statement about the common mode of action of local

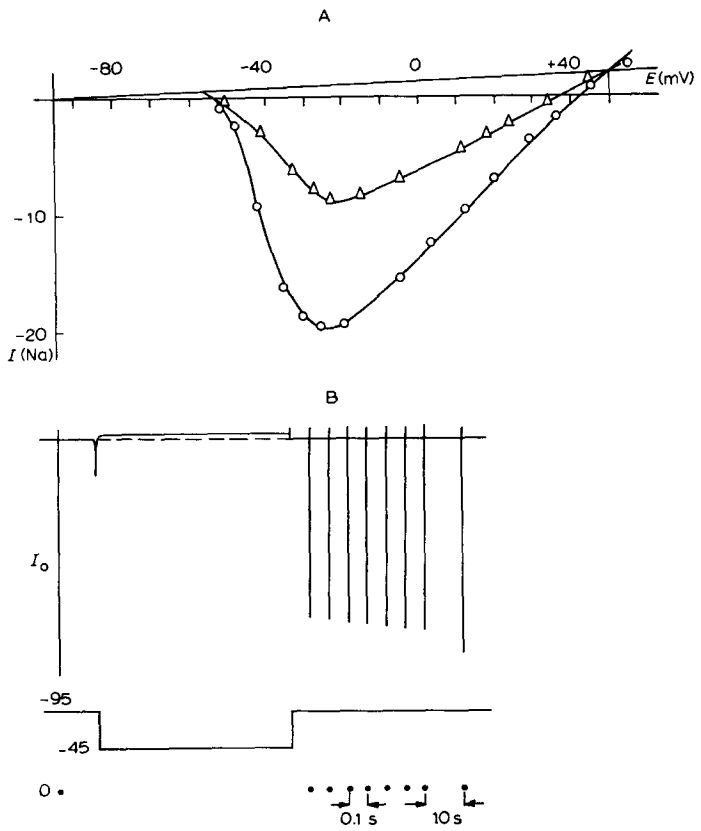

Fig. 16. Effects of internally applied trimecaine. A: current-voltage relations obtained 35 min after cutting the internode in isotonic KCl containing trimecaine (3.6 mM) before (open circles) and after (triangles) application of several 1 s long depolarizing pulses. B: sodium currents after a 1 s long depolarizing step to −45 mV. Test pulses to 0 mV are indicated by dots. Fibre 1.11.73.

anesthetics should be corrected. On the other hand a question arises whether slow inactivation contributes to frequency-dependent effects of externally applied tertiary anesthetics. It should be noted that in Strichartz's mechanism of use dependency a number of pulses is an important parameter while pulse duration appears to be more important in slow inactivation. With this in mind we carried out a study of sodium currents during repetitive stimulation under the action of externally applied procaine, trimecaine and QX-572 as well as under the internally applied procaine, trimecaine and QT (Figs. 16–18). The interval between the pulses in the series was 100 ms/period, quite sufficient for complete removal of fast sodium inactivation. On the external application of procaine and trimecaine we observed a fall of peak sodium currents in the sequence of pulses (Fig. 17A) characterized by the following properties.

(i) The relative value of the current drop increased with increasing duration of each pulse (Fig. 17B) and (ii) after the end of pulse sequence sodium currents were completely restored in the course of about 1 s, the restoration being of exponential time course with time constant equal to that of the restoration of currents inhibited by single prolonged depolarizing step (i.e. removal of slow inactivation) measured

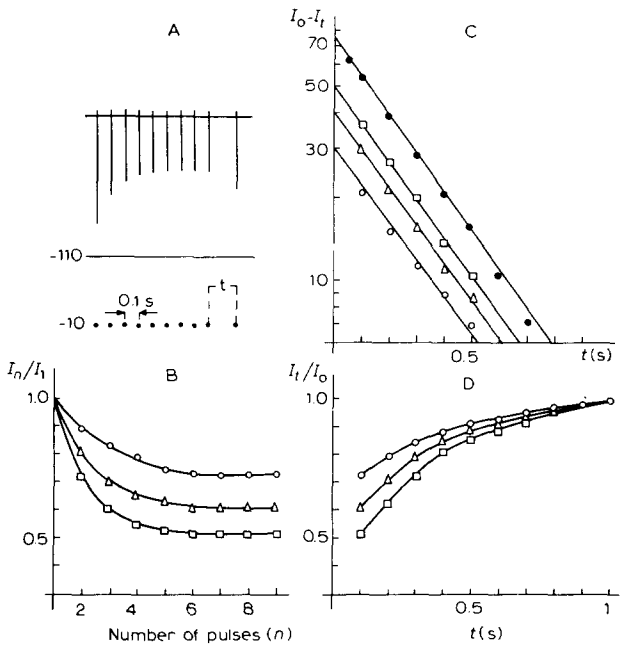

Fig. 17. Inhibition of sodium currents by repetitive stimulation in the node treated with externally applied trimecaine. A: original record showing the decrease of peak sodium currents in the series of 1 ms long test pulses to −10 mV going at the rate of 10 per s. B: peak sodium currents as a function of the number of pulses in the series plotted from the experiment illustrated in A; pulse durations: 1 ms (open circles), 5 ms (triangles) and 10 ms (squares). C: semilogarithmic plots of the time courses of recovery of sodium currents inhibited by series of short pulses shown in B (open circles, triangles, squares) and by single prolonged (1 s) depolarizing pulses (filled circles). D: same as in C but plotted in a linear current scale. Fibre 52.09.74.

in the same experiment (Fig. 17CD). These features strongly suggest that inhibition of currents during repetitive stimulation in the case of externally applied tertiary amine anesthetics is largely due to the accumulation of slow inactivation caused by each pulse. This conclusion is favored by the fact that the frequency-dependent inhibition is observed in the case of potassium-induced slow inactivation as well (external potassium concentration 25–50 mM). To make a quantitative estimate of the contribution of slow inactivation to the frequency-dependent inhibition we carried out the calculations using the model of slow inactivation (Eqns. 1–11) with rate constants τ_s^+ and τ_s^- measured in the same experiments. The experimental and calculated values of steady-state inhibition (attained by the tenth pulse in the series) are presented in Table VI. As one can see calculated values are very close to that observed in the experiments with variation of pulses duration (1–10 ms) and pH. According to the model the deepening of inhibition with the increase of pH is due to the influence of H^+ on slow inactivation kinetics (see Table V). So we conclude that under the experimental conditions used (high negative E_h) the inhibition of sodium currents during repetitive stimulation observed under the action of externally applied tertiary anesthetics was due mainly to slow inactivation.

On the other hand, inhibition of currents which we observed in case of QX-572

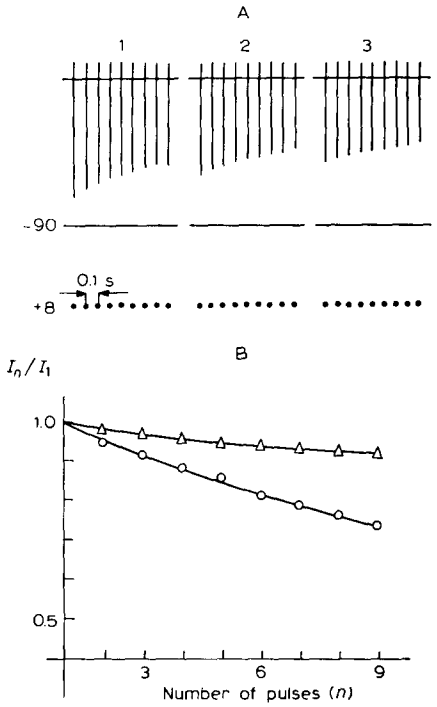

Fig. 18. Inhibition of sodium currents by repetitive short-term stimulation in the nodes treated with QX-572 (external application) and procaine (internal application). A: QX-572, pulse frequency; 10 per s, pulse duration; 1 (1), 5 (2) and 10 (3) ms. Fibre 25.01.75. B: peak sodium currents as a function of the number of pulses in the series. Circles plotted from the experiment shown in A. Triangles taken 35 min after cutting the internode in isotonic KCl with 3.6 mM of procaine. Some points for 1 and 5 ms pulse duration. Fibre 25.09.74.

TABLE VI

EFFECT OF pH ON THE USE-DEPENDENT INHIBITION OF SODIUM CURRENT

Fibre	Drug (mM)	pH	Duration of the pulse (ms)	I_n/I_1 ($n = 10$)	
				Observed	Calculated
30.01.75	Trimecaine 0.09	7.3	1	0.95	0.91
			5	0.87	0.83
			10	0.72	0.75
		5.6	1	0.99	0.98
			5	0.96	0.93
			10	0.92	0.86
29.01.75	Procaine 0.09	7.3	1	0.99	0.98
			5	0.95	0.94
			10	0.90	0.89
		8.5	1	0.99	0.94
			5	0.90	0.89
			10	0.82	0.84
26.01.75	Procaine 0.09	7.15	1	0.97	0.96
		8.5	1	0.89	0.86

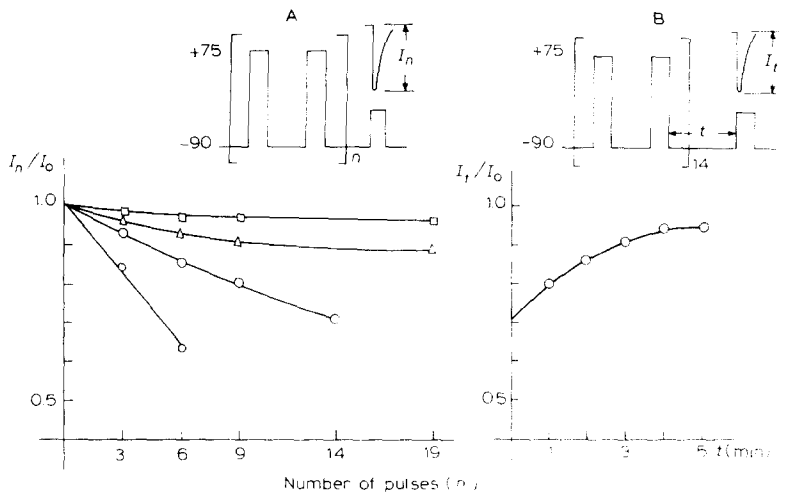

Fig. 19. Inhibition of sodium currents caused by repetitive stimulation of high amplitude. (to $E = +75$ mV). A: Top pattern of applied voltages. A number, n, of conditioning pulses with the duration of 5 ms are applied to the membrane before current, I_n, is measured during standard test pulse to -5 mV. All pulses are spaced 100 ms apart. Bottom: squares-internode cut in solution with 3.6 mM of trimecaine, fibre 1.11.73; triangles, internode cut in solution with 3.6 mM of procaine, fibre 18.10.73; circles, internally applied QT (0.25 mM), fibre 10.01.74; small circles, externally applied QX-572 (0.14 mM), fibre 21.01.75. B: time course of the recovery of sodium currents after series of 14 depolarizing pulses in the experiment with QX-572 shown in A. Top-pattern of applied voltages. Bottom, peak current on the test pulse applied at the moment t after the end of conditioning pulse series.

and internally applied tertiary anesthetics and QT showed no dependence on pulse duration (Fig. 18). This is exactly what Strichartz's model predicts, since according to it most of the blocking takes place in 1–2 ms with the onset of pulse. The striking feature of frequency-dependent inhibition produced by quaternary anesthetics was a very slow recovery of currents. In our experiments with QT the recovery of currents after 14 pulses to $+75$ mV took several minutes and was incomplete (Fig. 19). We cannot exclude that this mechanism contributes also to the use-dependent inhibition induced by externally applied procaine or trimecaine if the concentrations of these drugs are high enough (> 0.5 mM) and E_h is near the level of normal resting potential (~ -70 mV). Under such condition slow inactivation mechanism will be nearly saturated ($s_\infty \to s_\infty^{\min}$) and can be only partially responsible for use-dependent blockage of sodium channels. It is necessary to emphasize that Hille et al. [29] and Courtney [31] have employed a relatively high concentrations of lidocaine and GEA-968 and that they clamped membrane at the level of resting potential.

DISCUSSION

Common to all local anesthetics is their ability to cause a block of the sodium permeability which cannot be removed by hyperpolarization. In terms of the Hodgkin-Huxley model this corresponds to a decrease of the sodium permeability parameter $\bar{P}_{Na}$. Between the anesthetics, however, there are considerable differences with respect

to the mechanism of this potential independent block and their ability in addition to the influence on $\bar{P}_{Na}$, to cause a potential dependent block of sodium permeability such as slow inactivation and use-dependent inhibition described by Strichartz [13]. The differences in the mechanism of the reduction of $\bar{P}_{Na}$ is most clearly manifested by comparing the effects of benzocaine and quaternary derivatives, namely, QX-314, QX-572 and QT. Judging by findings [29, 31] and the results of the present study, permanently charged quaternary compounds exert a direct blocking action on sodium channels by binding to the receptor (R_3) located at the internal opening of the channel. Neutral benzocaine which does not dissociate at pH 7 is evidently incapable of interacting with this receptor. As we have already mentioned many researchers assume that the decrease of P_{Na} caused by procaine, trimecaine and other tertiary amines is based on the same mechanism as the action of quaternary derivatives, i.e. the block of the internal openings of sodium channels by charged molecules of this drugs. However there are some data which indicate the resemblance of the mechanism of P_{Na} decrease caused by procaine and trimecaine upon external application to that of benzocaine: (i) the dose-effect curves for trimecaine and benzocaine are similar and are characterized by about the same value of dissociation constant, and (ii) neither short nor prolonged depolarization enhance the effect of externally applied procaine, trimecaine and benzocaine on $\bar{P}_{Na}$ unlike that which takes place under the action of QT, QX-572 and QX-314. This is difficult to explain on the basis of the hypothesis of the direct block of internal openings of the sodium channels by protonated molecules of anesthetics. However additional studies are required for the final conclusion. A test of the effects of benzocaine on sodium currents in the node treated with batrachotoxin may prove to be useful. Earlier [32] we have found out that sodium channels modified by batrachotoxin are deprived of inactivation, both fast and slow, and are ten times less sensitive to the blocking action of procaine compared with normal sodium channels. Since in the absence of inactivation a drop in peak sodium currents means a decrease in P_{Na}, there is every reason to regard the competitition between procaine and batrachotoxin as a competition for the receptor R_1. If benzocaine acts on batrachotoxin-treated membrane like procaine, this may be regarded as an indication that the decrease in $\bar{P}_{Na}$ caused by these anesthetics is effected through the very same receptor R_1.

Slow sodium inactivation, which we regard as a result of drug molecule binding to some receptor (R_2) accessible in the inactivated channel, can be produced only by tertiary amine anesthetics applied from outside. Neither neutral benzocaine nor permanently charged quaternary derivatives of trimecaine QT and lidocaine QX-572 induce slow inactivation. Tertiary amine anesthetics applied from inside were also incapable of producing slow inactivation. From this it can be supposed that the receptor site R_2 has a specific affinity for tertiary nitrogen and is accessible from the outer side or the membrane. To decide the question about the active form of anesthetics interacting with R_2 the model is needed which would satisfactorily describe the effects of pH on slow inactivation kinetics and its steady state level.

The question could arise whether what we define as the reduction of $\bar{P}_{Na}$ may be due to the operation of the same mechanism which underlies the slow sodium inactivation. This may be the case if a certain fraction of sodium channels is normally inactivated in the untreated fibre even at high negative potentials (rate constant β_h of the transition to the inactive state is not zero) where h_∞ is assumed to be 1. Then

upon application of anesthetic a finite fraction of sodium channels will be transfered into the slow inactive state. However, according to quantitative estimates more than 10 per cent of the total number of sodium channels are required to be normally inactivated at high negative E values for the slow inactivation to account for the reduction of P_{Na} after the treatment with procaine or trimecaine. Though not completely incredible this figure seems to be too high.

The unremovable block in question cannot be attributed to the operation of frequency-dependent mechanism described by Strichartz since, as we have seen its contribution to the blocking action of externally applied tertiary anesthetics is immeasurably small even during repetitive stimulation.

Hille et al. [29] and Courtney [31] consider that the mechanism of frequency-dependent inhibition can be useful in the design of antiarrhythmic agents which limit repetitive activity to chosen maximum frequency. We suggest that slow inactivation may prove to be still another important tool for this purpose. The long lasting plateau of the action potential of cardiac muscle fibre is the condition ensuring inhibiting efficiency of slow inactivation mechanism and its effectiveness in prolongating the relative refractory period [33]. In summary we can draw the following conclusions. The membrane of the Ranvier node has several types of sites through which local anesthetics exert their blocking action on sodium permeability. Receptor R_1 seems to react with an uncharged molecule of tertiary amines local anesthetics and neutral benzocaine. Binding of R_1 leads to the fall of P_{Na}. Receptor R_2 is responsible for slow sodium inactivation and is accessible in the inactivated sodium channel presumably from the outer side of the membrane. R_2 interacts only with tertiary amines, their active form being unknown. Binding of local anesthetics to R_2 appears to be strongly affected by pH. Receptors R_3 and R_4 responsible for the "tonic" and "phasic" components of use-dependent inhibition, respectively, interact with permanently charged quaternary or protonated tertiary amines anesthetics and are located within the channel near its internal mouth. Little is know as yet about the chemical structure, stereoselectivity [29, 34] and other properties of these receptors. Still unclear are the causes of slow inactivation kinetics changes following shifts of pH of external solution. On the solution of this problem depends not only the determining of the active form of anesthetics but also the learning processes underlying slow inactivation. Of importance is the study of reasons why tertiary anesthetics, procaine and trimecaine, do not produce slow inactivation on their internal application. It is not clear why lipid-soluble molecules of these anesthetics in the form of free base cannot diffuse from axoplasm and reach the receptor R_2. Of great interest is a comparative analysis of the action of various local anesthetics on the sodium channels in the membrane treated with batrachotoxin, aconitine, veratridine and scorpion venom since, these drugs markedly change the gating function. Useful information may be obtained from the study of gating currents under the action of various local anesthetics. As yet it is known that procaine at relatively high concentration (1 %) reduces gating current in the squid giant axon [35].

ACKNOWLEDGEMENTS

We are pleased to thank Dr. E. Timin for valuable discussion and help in examining various models of pH effects on slow inactivation.

We are greatly indebted to Astra Pharmaceutical Company for a gift of QX-572. The quaternary derivative of trimecaine was generously donated by Dr. M. Kusnetzova of the Pharmacological Institute of Acid. of Medical Sciences, U.S.S.R.

REFERENCES

1 Weidmann, S. (1955) J. Physiol. 129, 568–582
2 Khodorov, B. and Belyaev, V. (1955) Biofizica, 10, 625–633
3 Khodorov, B. and Belyaev, V. (1967) Biofizica, 12, 855–863
4 Khodorov, B. (1974) The Problem of Excitability, pp. 115–120, Plenum Press, New York
5 Belyaev, V. (1973) in Biofizica Membranes, 76–81, Kaunas
6 Narahashi, T. (1964) J. Cell. Comp. Physiol. 64, 73–96
7 Taylor, R. (1959) Am. J. Physiol. 196, 1071–1078
8 Shanes, A., Freygang, W., Grundfest., H. and Amantiek, R. (1959) J. Gen. Physiol. 41, 793–802
9 Narahashi, T., Moore, J. and Poston, R. (1969) J. Neurobiol. 1, 3–22
10 Hille, B. (1966) Nature 210, 1220–1222
11 Peganov, E., Khodorov, B. and Shishkova, L. (1973) Bull. Exp. Biol. Med. 9, 15–19
12 Khodorov, B., Shishkova, L., and Peganov E. (1974) Bull. Exp. Biol. Med. 3, 10–13
13 Strichartz, G. (1973) J. Gen. Physiol. 62, 37–57
14 Khodorov, B., Shishkova, L., Peganov, V. and Revenko, S. (1975) in Physiological Role of Surface Active Substances, pp. 7–10, Tchernovitzi
15 Khodorov, B. (1975) General Physiology of Excitable Membranes, Nauka, Moscow
16 Dodge, F. and Frankenhaeuser, B. (1958) J. Physiol. 143, 76–90
17 Prjanishnikova, N. and Sharov, N. (1967) Trimecaine Pharmacology and Clinical Use, Medicina, Leningrad
18 Adelman, W. and Palti, J. (1969) J. Gen. Physiol. 54, 589–606
19 Arhem, P. and Frankenhaeuser, B. (1974) Acta Physiol. Scand. 91, 11–21
20 Hodgkin, A. and Huxley, A. (1952) J. Physiol. 117, 500–544
21 Goldman, D. (1943) J. Gen. Physiol. 27, 37–60
22 Dodge, F. (1962) Proc. of IUPS, 22nd Int. Congr., Leden, p. 573.
23 Blaustein, M. and Goldman, D. (1966) J. Gen. Physiol. 49, 1043–1063
24 Hille, B. (1968) J. Gen. Physiol. 51, 221–236
25 Feinstein, M. and Paimer, M. (1966) Biochim. Biophys. Acta 115, 33–45
26 Kwant, W. and Seeman, P. (1966) Biochim. Biophys. Acta 193, 338–349
27 Kholodova, J. and Sorokina, Z. (1971) in Biofizica Membranes, 768, Kaunas
28 Narahashi, T. and Frazier D. (1971) Neurosci. Res. 4, 65–99
29 Hille, B., Courtney, K. and Dum, R. (1975) in Molecular Mechanism of Anesthesia, Issaquah, Progress in Anesthesiology, Vol. 1, pp. 13–24, Raven Press, New York
30 Belyaev, V. (1973) Bull. Exp. Biol. Med. 12, 47–50
31 Courtney, K. (1974) Frequency Dependent Inhibition of Sodium Currents in Frog Myelinated Nerve by GEA 968, A New Lidocaine Derivative, Dissertation, Seattle, U.S.A.
32 Khodorov, B., Peganov, E., Revenko, S. and Shishkova, L. (1975) Brain Res. 84, 541–546
33 Haas, H., Kern, R., Einwachter, H. and Tarr, M. (1971) Pfluger's Arch. 323, 141–157
34 Åkerman, B. (1973) Studies on the Relative Pharmacological Effects of Enantiomers of Local Anesthetics with Special Regard to Block of Nervous Excitation, Uppsala
35 Keynes, R. and Rojas, E. (1974) J. Physiol. 239, 393–434