BLOCK AND INACTIVATION OF SODIUM CHANNELS IN NERVE BY AMINO ACID DERIVATIVES

I. Dependence on Voltage and Sodium Concentration

Mei-Ven C. Lo and Peter Shrager, *Department of Physiology, University of Rochester Medical Center, Rochester, New York 14642*

ABSTRACT The side chain of arginine, *n*-propylguanidinium (*n*PG), reversibly decreases peak sodium conductance and increases the speed of sodium current decay, when perfused internally. Effects are voltage dependent and are more pronounced at high depolarizations. Results are also dependent on the sodium concentration gradient. Both the decline in peak conductance and the speeding of inactivation are greater if the sodium concentration gradient is reversed from the normal. The decrease in peak sodium current is too large to be due solely to the faster decay kinetics. The difference is not due to a change in slow inactivation of the channel. Sodium current inactivation has also been studied with a double pulse procedure. Results show that at −70 mV, *n*PG leaves sodium channels rapidly (<500 μs) in normal sodium gradient, but more slowly (>1 ms) in reversed sodium gradient. Several structural analogs of *n*PG have been tested. Shortening the alkyl chain weakens effects significantly. Arginine itself, which contains extra charged groups, is also less effective. *n*-Propylammonium is active but with an apparent affinity only one-fifth that of arginine. We conclude that *n*PG acts within the sodium channel, and has at least two modes of action.

INTRODUCTION

In normal nerve fibers sodium channels open in response to a depolarization and then close spontaneously. This latter process is known as inactivation and was first described by Hodgkin and Huxley (1952 *a*). A large number of recent studies have focused on the mechanism of inactivation and have provided some clues to the chemical structures and events involved. Armstrong et al. (1973) have shown that internal perfusion with pronase, a mixture of proteolytic enzymes, results in elimination of inactivation. Rojas and Rudy (1976) found that the active component of pronase is alkaline proteinase b, an endopeptidase with a specificity close to that of trypsin, i.e., hydrolysis of the peptide bond between the carboxyl group of arginine or lysine and the amino group of the neighboring amino acid. These authors showed further that after exposure to the enzyme, perfusion with certain quaternary ammonium compounds could restore a time-dependent closing of sodium channels. A reasonable tentative conclusion was that pronase removed or disrupted a cationic peptide and that other organic cations might be able to substitute for it.

This idea has been strengthened by work in other laboratories. Yeh and Narahashi (1977) found that pancuronium, a quaternary ammonium steroidal compound, speeds inactivation of sodium channels and restores inactivation after pronase exposure. Yeh and Armstrong (1978) later showed that pancuronium also induced immobilization of gating charge, an event associated with normal inactivation (Bezanilla and Armstrong, 1977; Armstrong and Bezanil-

la, 1977). Quaternary derivatives of local anesthetics and of strychnine also block sodium channels and immobilize gating charge, and these drugs appear to compete with the inactivation process (Cahalan and Almers, 1979 *a*,*b*).

The above cationic blockers bear no obvious relation to biochemical structures that are thought to be present normally in nerve membranes. Eaton et al. (1978), however, investigated the possible involvement of several amino acids in sodium channel inactivation. These authors found that chemical modification of squid axon membranes by reagents known to block arginyl residues prevented a significant fraction of sodium channel inactivation. They tested a number of free amino acids for their ability to reduce Na^+ currents in axons pretreated with pronase. Of the compounds tested only arginine produced block, but the effect was small: 50 mM arginine internally blocked 24% of I_{Na}. Eaton et al. (1978) also found that polyglycyl arginine amide blocked Na^+ currents in a time-dependent manner in these fibers.

In this and the following paper (Lo and Shrager, 1981) we report experiments designed to test further the idea that a cationic amino acid may be involved in inactivation. In particular, we have tested the side chain of arginine, *n*-propyl guanidinium (*n*PG), under a variety of experimental conditions. In this paper we examine the alteration of peak Na^+ currents and decay kinetics by the drug and the dependence of these phenomena on voltage and on Na^+ concentrations. In the next paper we present the dependence of *n*PG action on temperature and drug concentration, and give a detailed kinetic model to account for our observations.

A brief report of this work has been presented to the Biophysical Society (Lo and Shrager, 1980). Concurrent with these studies, experiments with long chain (C_8, C_9) alkyl guanidines in squid axons have been performed in two laboratories (Kirsch et al., 1980; Morello et al., 1980).

METHODS

The Axon

Medial giant axons from the crayfish *Procambarus clarkii* were internally perfused and voltage clamped as previously described (Shrager, 1974; Starkus and Shrager, 1978). Briefly, cleaned axons were cannulated with an axial wire-potential pipette "piggy-back" electrode, and, from the opposite end, a perfusion inlet pipette. The insulated electrode shank was kept small to allow the internal perfusate to flow out through the electrode entry hole. The external current measuring system consisted of a 1-mm central region and two 4-mm guard sections; the entire assembly was shaped to surround the axon over 270° of its circumference, at a distance of about 200 μm. A reference potential pipette was placed close to the fiber in the central region. Compensation for series resistance was used, with an estimated 1–2 Ω cm^2 remaining uncompensated. Temperature was controlled by Peltier devices and a feedback circuit to within ± 0.1°C.

Voltage clamp pulses were generated by a fast 12-bit deglitched, D/A converter controlled by a PDP 8/A-620 computer (Digital Equipment Corp., Maynard, Mass.). A preliminary subtraction of capacitative transients and leak currents was made electronically and current signals were then fed to a sample and hold amplifier and 12-bit A/D converter. Sampling rate varied from 5 to 100 μs/point. Data were stored on magnetic disks for analysis.

Final subtraction of capacitative transients and leak currents was achieved by scaling a hyperpolarizing pulse. Peak currents were measured by fitting records in the peak region with a third-order polynomial, solving for the extremum, and monitoring the fit visually (Hille, 1971). In some cases, Na^+ currents did not inactivate completely and a small residual steady-state current was left. This value was subtracted from the record before fitting single exponentials to decays. Fits were made using a least-squares procedure with weighted residuals, and were monitored visually.

TABLE I
SOLUTION COMPOSITIONS

Name	External solutions (*mM*)							
	Na^+	K^+	Mg^{++}	Ca^{++}	TMA^{+*}	Cl^-	HCO_3	pH
NVH	207.3	5.4	2.6	13.5		242.6	2.3	7.6
Na2.3VH	2.3		2.6	13.5	217.4	249.6	2.3	7.6
K1.0VH	211.7	1.0	2.6	13.5		242.6	2.3	7.6

	Internal solutions (*mM*)						
	K^+	Na^+	F^-	$Citrate^{-3}$	Cl^-	Mannitol	pH
Pl	220	15	109	37	15	93	7.35
Na235		235	109	37	15	93	7.35
Na213	22	213	109	37	15	93	7.35

*TMA, tetramethylammonium.

Solutions and Reagents

The standard external solution (NVH) used for dissection in all experiments was that of Van Harreveld (1936). The standard internal perfusate (Pl) and all other solutions used are listed in Table I. In the text, solutions are given as external//internal. *n*PG $(sulfate)_{1/2}$ and methylguanidinium chloride were obtained from Vega Biochemicals, Tuscon, Ariz. Experiments in "normal" $[Na^+]$ gradient were made in K1.0VH//Pl with 1.5–2.0 mM 4-aminopyridine added to both solutions to block K^+ currents (Meves and Pichon, 1975; Yeh et al., 1976) and 2 mM Hepes buffer added externally (pH 7.6). Axons in "reversed" $[Na^+]$ gradient were in Na2.3VH//Na235 (or, where noted, Na2.3VH//Na213).

RESULTS

The structures of some of the compounds used in this study are shown in Fig. 1. Results of adding 4.4 mM *n*PG to the internal perfusate are illustrated in Fig. 2. In the experiment of

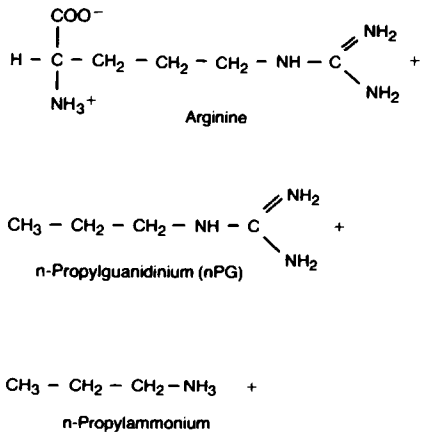

FIGURE 1 Chemical structures of three compounds used in this study. For the guanidinium group in arginine the pK = 13.2, whereas the pK for alkyl amino groups is ~10. Thus, these molecules are almost entirely in the cationic form at physiological pH.

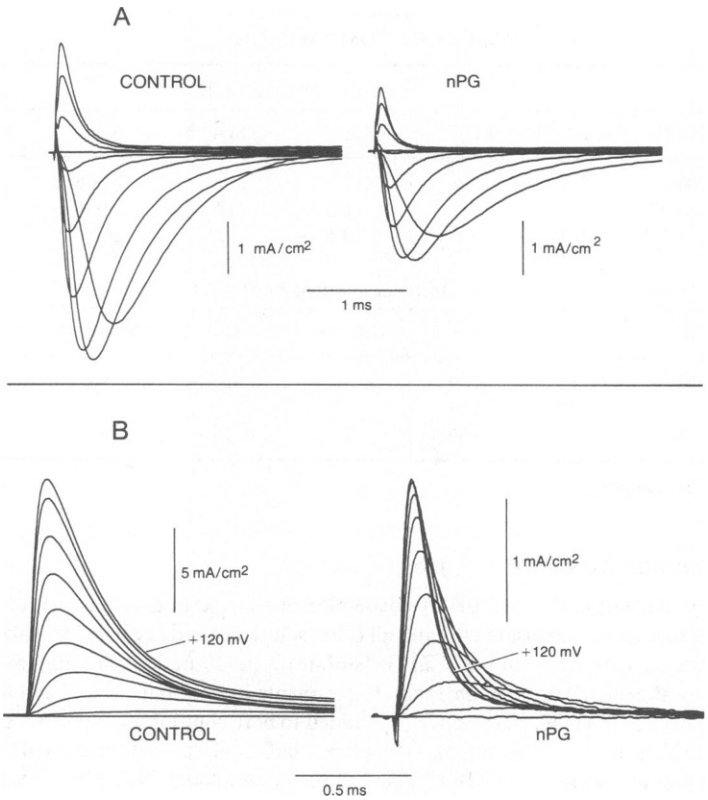

FIGURE 2 The action of *n*PG on sodium currents. *A*, Axon in normal [Na^+] gradient. K^+ currents were reduced by 2 mM 4-aminopyridine and eliminated by subtracting records in 100 nM TTX. Internal [*n*PG] = 4.4 mM. Depolarizations were from the holding potential of −70 mV to −30, −20, −10, +10, +30, +50, +70, +90, and +110 mV. Temperature = 8°C. *B*, Axon in reversed [Na^+] gradient. Capacitative transients and linear leakage currents have been subtracted by scaling the response to a hyperpolarizing pulse. Internal [*n*PG] = 4.4 mM. Holding potential = −50 mV. A 100-ms prepulse to −110 mV was separated by a return to the holding potential for 0.5 ms from test depolarizations to −30, −10, +10, +30, +50, +70, +90, +110, and +120 mV. Note large difference in current scale in *n*PG. Temperature = 6°C.

Fig. 2 *A* the fiber was initially perfused externally and internally with K1.0VH//P1. 2 mM 4-aminopyridine (4-AP) was added to both solutions to block most of the potassium current (Meves and Pichon, 1975; Yeh et al., 1976) and a set of records was obtained. We applied 4.4 mM *n*PG internally and recorded another current family. Tetrodotoxin (TTX, 100 nM) was then added externally and records were made before and after wash-out of the *n*PG. The families shown in Fig. 2 *A* represent sodium currents, obtained by subtracting sweeps with TTX from those without, in the computer. Because of both the length of time and the exposure to *n*PG (which altered I_K somewhat) the TTX subtraction procedure was not entirely satisfactory in the control family, particularly at low depolarizations, but this method was necessary for reliable measurements of decay time constants at higher depolarizations. *n*PG shifted the zero current (reversal) potential to lower values. This can be seen qualitatively in Fig. 2 *A*. From currents at higher amplification, and with depolarizations

spaced just a few millivolts apart, we measured reversal potentials of +57 mV (control) and +52 mV (*n*PG). This change of 5–6 mV with 4–5 mM *n*PG was a consistent finding. Calculation of peak sodium conductances (g_P) from the data of Fig. 2 *A* showed them to be reduced to 0.6 of control values at all V_m. The decay time constant of sodium current was little affected at lower depolarizations, but was speeded by 40% at higher voltages. Small differences in decay kinetics at low depolarizations were accountable in terms of the remaining uncompensated portion of the series resistance (see Methods) and the steepness of the τ-V_m relation. Tested on a single intact axon, 4.4 mM *n*PG applied externally for 45 min had no effect on either peak Na^+ currents or decay time.

Effects of *n*PG were significantly stronger when the Na^+ concentration gradient was reversed. An example is shown in Fig. 2 *B*. After dissection and positioning of electrodes, solutions were changed to Na2.3VH//Na235 and control currents were recorded. Below −30 mV in these solutions, sodium currents were too small to be reliable. 4.4 mM *n*PG was then added internally, and the protocol was repeated. Peak currents were reduced in a voltage-dependent manner, with increased block at stronger depolarizations. I_P in *n*PG was only one-fifth of control levels at the most positive potentials. The decay time constant of I_{Na} was faster with *n*PG present and was also reduced more at higher depolarizations. Note the position of the curve at 120 mV in the *n*PG family. Effects of *n*PG were almost completely reversed on washing. Fig. 3 illustrates results from another fiber, in which all currents are plotted to the same scale. Reversal potentials could not be accurately determined in reversed $[Na^+]$ gradient. However, test depolarizations were sufficiently far from the expected reversal potential so that a small shift would produce little error in calculating conductances.

Decay time constants were calculated by fitting single exponentials to the data, using a weighted least-squares procedure. The fit was generally good, as shown in Fig. 4, although at the highest depolarizations, another much slower component was often seen in both control and *n*PG records. In these latter cases, deviation from a good fit occurred when the decay was about 90% complete, and this small component was ignored (see also Bean, 1979). Decay time constants and their ratio are plotted vs. membrane potential in Fig. 5 (top). The reduction of τ by *n*PG is clearly not the result of a simple shift along the voltage axis. Similarly, the decrease

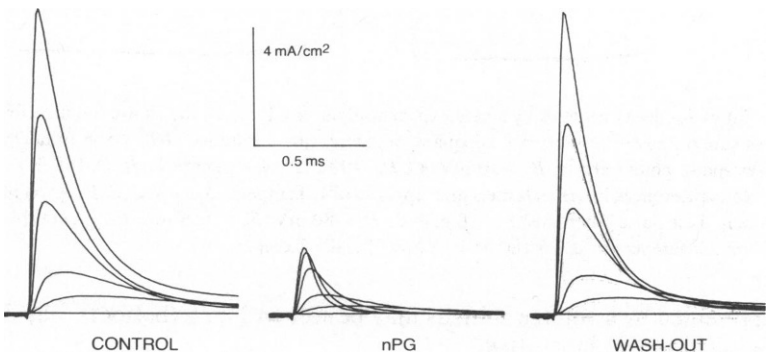

FIGURE 3 Reversibility of *n*PG effects. Axon in reversed $[Na^+]$ gradient. Capacitative transients subtracted by scaling a hyperpolarizing pulse. Holding potential = −38 mV. A 50-ms prepulse to −118 mV preceded test depolarizations to −18, +2, +42, +82, and +122 mV. All records at the same scale. Internal [*n*PG] = 4.4 mM. Temperature = 8°C.

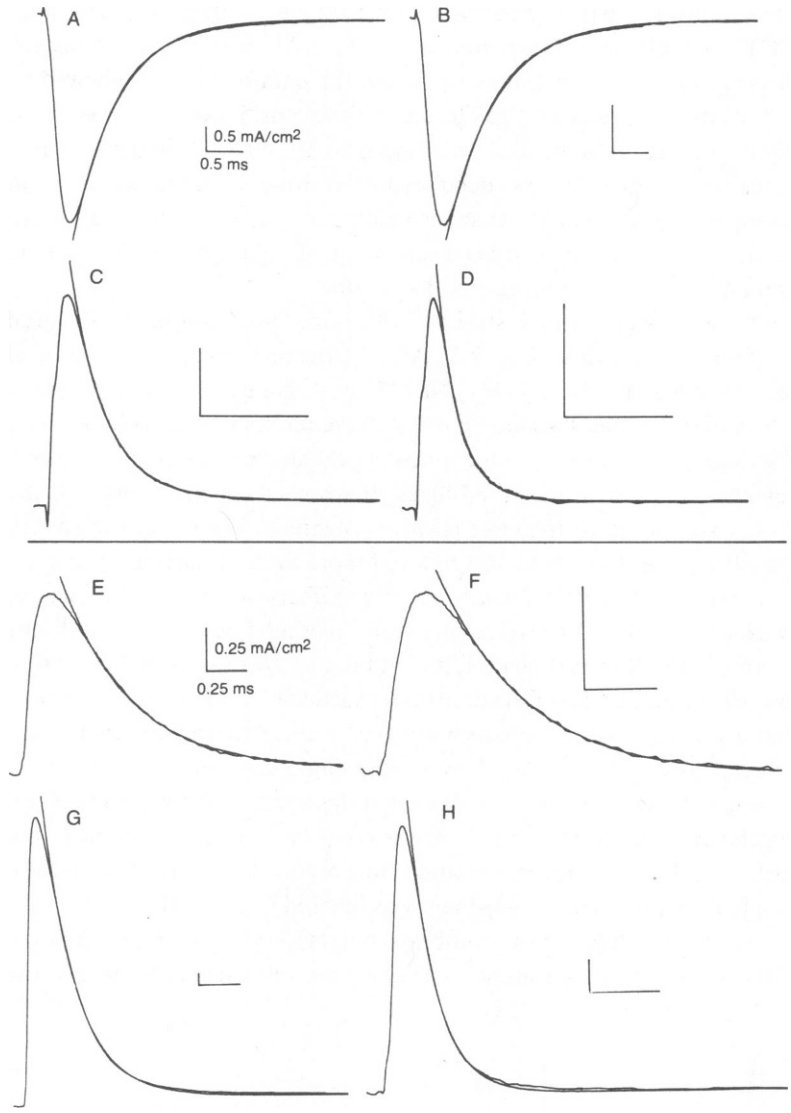

FIGURE 4 Fit of I_{Na} decay kinetics by single exponential curves. Each frame shows sodium currents and fitted curves superimposed, to illustrate adequacy of single time constants. *A-D*, Axon in normal [Na^+] gradient. Test-pulse potentials: *A*, *B*, −20 mV; *C*, *D*, +90 mV. *A*, *C*, controls; *B*, *D*, [*n*PG] = 4.4 mM internally. Note differences in scales (labels in *A* apply to all). Temperature = 8°C. *E-H*, Axon in reversed [Na^+] gradient. Test-pulse potentials: *E*, *F*, 0 mV; *G*, *H*, +80 mV. *E*, *G*, controls; *F*, *H*, [*n*PG] = 4.4 mM internally. Note differences in scales (labels in *E* apply to all). Temperature = 8°C.

in I_P is not represented by a voltage shift, as may be seen in Fig. 5 (bottom). Fig. 5 shows data for an axon in reversed [Na^+] gradient.

Peak Sodium Conductance

The decrease in peak current is greater than that expected from the faster τ alone. As one test, sodium conductance was fit by $\bar{g}_{Na}m^3h$ kinetics (Hodgkin and Huxley, 1952 *b*) where $\bar{g}_{Na}$ is

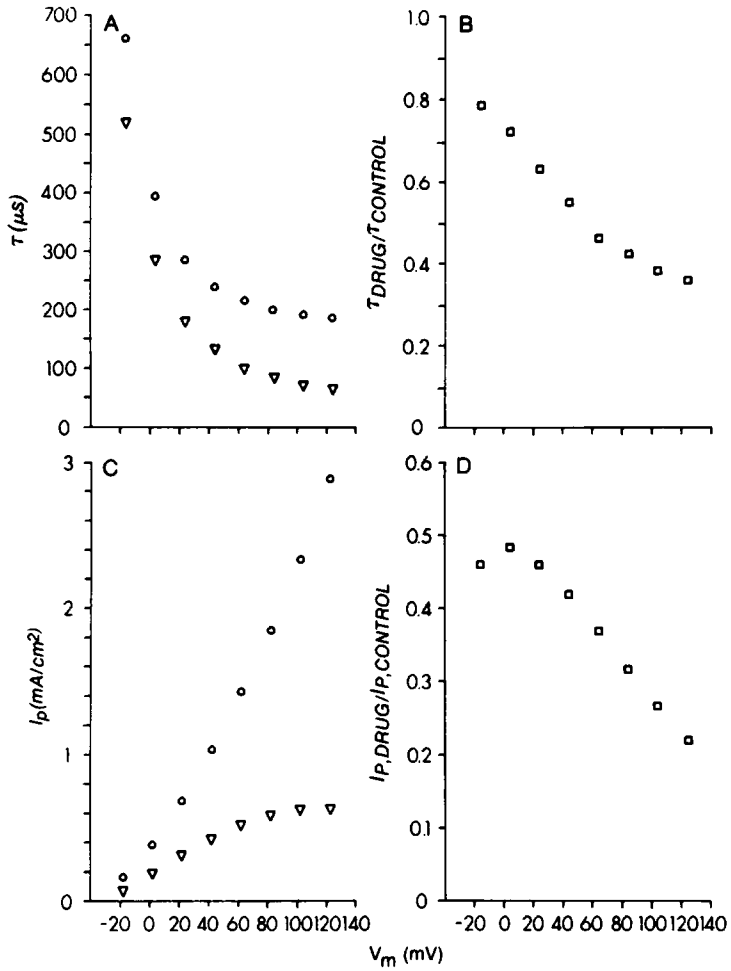

FIGURE 5 Reduction of decay time constant and peak current by *n*PG. Axon in reversed [Na^+] gradient. *A*, time constants vs. membrane potential. No drug (circles) and with internal [*n*PG] = 4.4 (triangles). *B*, ratio of time constants with and without *n*PG. *C*, peak currents before (circles) and after (triangles) internal application of 4.4 mM *n*PG. *D*, ratio of peak currents with and without *n*PG. Temperature = 8°C.

the maximum sodium conductance with all channels open, and m and h are probability variables representing independent activation and inactivation, respectively (applicability of coupled vs. independent models will be discussed later).

The computer first fit control and *n*PG records, providing values of τ_m and τ_h for each. The fit was judged by viewing actual and calculated curves superimposed on the oscilloscope, and was generally satisfactory, but with several limitations: (*a*) nonexponential decay at the highest depolarizations as discussed earlier, (*b*) reversal potential not measurable in reversed [Na^+] gradient, and (*c*) clamp speed and digital sampling rate are marginally adequate at the fastest τ_m (~20 μs). Conductances were then calculated using the $\bar{g}_{Na}$ of control fits and the time constants from *n*PG records, and the peaks were found. Calculated peak conductance

TABLE II

PEAK SODIUM CONDUCTANCE: EXPERIMENT VS. CALCULATIONS WITH CONSTANT $\bar{g}_{Na}$

[Na^+] gradient	Control or *n*PG	V_m	τ_h	τ_m	$g_{p,nPG}/g_{p,control}$	
					Calculated	Experimental
Reversed	Control	0	369	93	0.98	0.33
	*n*PG	0	294	77		
	Control	+40	221	43	0.83	0.29
	*n*PG	+40	143	38		
	Control	+80	188	27	0.78	0.23
	*n*PG	+80	95	22		
Normal	Control	−10	487	85	1.09	0.58
	*n*PG	−10	496	85		
	Control	+30	258	50	1.21	0.57
	*n*PG	+30	221	44		
	Control	+90	155	32	0.61	0.54
	*n*PG	+90	90	32		

The conductance ratios in the normal [Na^+] gradient are calculated with measured reversal potentials: control, +57 mV; *n*PG, +52 mV. The ratios in reversed [Na^+] gradient are calculated assuming reversal potentials are unchanged by *n*PG. Since $V_m - V_{rev}$ is large at all V_m, a small change in V_{rev} would introduce littler error.

ratios are compared with experimental values in Table II. Four experiments were analyzed and data from two are given in the table for a few membrane potentials. Results from the other fibers were identical. In all cases, conductance ratios calculated assuming constant $\bar{g}_{Na}$ are higher than actual ratios. Further, in the reversed [Na^+] gradient experiment this is not due solely to the somewhat faster τ_m values in *n*PG. Calculated peak ratios using control τ_m and nPG τ_h were 0.85, 0.76, and 0.68 at 0, +40, and +80 mV, respectively. Therefore, even with the limitations discussed above, it is clear from the data that the measured conductance ratio is too small to be due only to the faster decay τ. *n*PG thus seems to reduce peak g_{Na} by at least two mechanisms.

One possible process that might contribute to lowered peak currents is "slow" or "long-term" Na^+ inactivation. Crayfish axons have significant slow inactivation that develops through the holding potential range −80 to −50 mV (Starkus and Shrager, 1978). Further, various reagents have been found that either close or open slow inactivation gates (Shrager, 1977; Starkus and Shrager, 1978). Effects of *n*PG, however, are not due to significant modification of this system. In the experiment illustrated in Fig. 6, an axon in reversed [Na^+] gradient was held at either −50 mV or −70 mV and currents were recorded before and after adding 2 mM *n*PG internally. Results were virtually unchanged despite the fact that slow inactivation is ~10 times greater at −50 mV than at −70 mV (Starkus and Shrager, 1978). Differences between Figs. 6 and 5 are due to different *n*PG concentrations used (2 and 4.4 mM, respectively, see also Lo and Shrager, 1981). In Table III we show another way of examining slow inactivation. Prepulses to −110 mV were applied for durations varying from 50 to 1400 ms. V_m was then returned to the holding potential for 0.5 ms before applying a test depolarization and measuring peak sodium current. From these numbers we calculate the ratio of peak currents with prepulse durations of 50 and 350 ms, and also calculate time constant of recovery from slow inactivation (τ_s). This peak current ratio is a rough indicator of

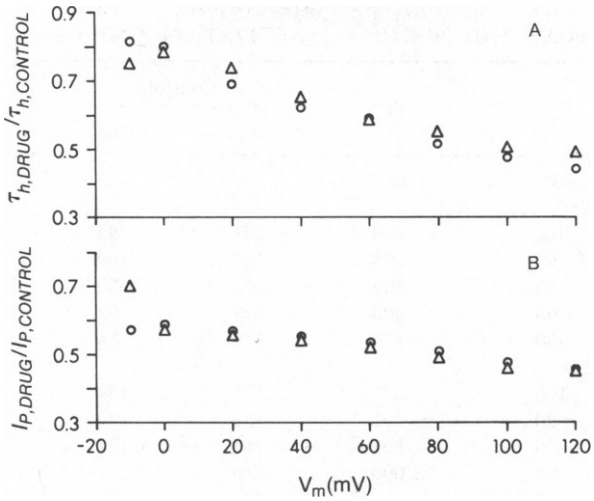

FIGURE 6 *n*PG action is independent of holding potential. Axon in reversed [Na^+] gradient. Ratio of sodium current decay time constants (*A*) and of peak currents (*B*) with and without 2 mM *n*PG internally. Δ, V_H = −50 mV; ○, V_H = −70 mV. In all records a 50-ms prepulse to −110 mV was separated by a return to the holding potential for 0.5 ms from test depolarizations. This prepulse is sufficient to eliminate most "fast" sodium inactivation, but has little effect on slow inactivation. Temperature = 8°C.

steady-state slow inactivation since fast inactivation (h) is complete in 50 ms and the slow inactivation time constant is <200 ms at −110 mV (Starkus and Shrager, 1978). The table shows that *n*PG has little effect on either steady-state or kinetic properties of slow inactivation. We conclude that the reduction in peak I_{Na} by *n*PG (in excess of that caused by the faster decay time) is not due to an increase in slow inactivation.

Decay Kinetics

The kinetics of inactivation were studied further using a double-pulse procedure. Prepulses to different potentials, and of varying durations, were separated in time from test pulses by a return to the holding potential for 200–1,000 μs (T_2). Time constants (τ_c) were measured from the decrease in peak current during the test pulse as the duration of the prepulse was

TABLE III
SLOW INACTIVATION OF SODIUM CHANNELS

Axon	[Na^+] gradient	V_H*	V_{PP}	$I_{P,50}/I_{P,350}$		τ_s	
				Control	*n*PG	Control	*n*PG
		mV	*mV*			*ms*	*ms*
1	normal	−70	−110	0.68	0.64	190	158
2	reversed	−50	−110	0.23	0.17	145	152

*V_H, holding potential; V_{PP}, prepulse potential. Test-pulse potentials: axon 1, +10 mV; axon 2, +30 mV. $I_{P,50}/I_{P,350}$ = ratio of peak current with prepulse of 50 ms to that with prepulse of 350 ms. τ_s, time constant of recovery from slow inactivation. Temperature, 6°C.

TABLE IV

I_{Na} DECAY AND PREPULSE INACTIVATION TIME CONSTANTS

Axon	V_m	T_2	Control		*n*PG	
			τ_h	τ_c	τ_h	τ_c
	mV	μs	μs	μs	μs	μs
Normal Na Gradient						
N 1	+87	500	181	192	*	172
N 2	+90	500	160	169	92	154
	−10	500	523	650	497	597
N 3	+90	500	159	179	97	170
	+90	200	159	156	97	132
Reverse Na Gradient						
R 1	+100	200	175	144	77	86
R 2	+90	200	186	122	64	66
	+90	500	186	133	64	59
	+90	1,000	186	132	64	58
	−10	200	447	499	324	325
	−10	500	447	494	324	408
	−10	1,000	447	498	324	418

I_K reduced by 1–2 mM 4-aminopyridine. R 2 at 11°C; all others at 8°C.

*I_K excessive: τ_h could not be accurately determined.

varied. These values are compared with the time constant of decay of I_{Na} (τ_h) (Goldman and Schauf, 1973) in Table IV. The voltages studied are in the range in which *n*PG has significant effects, and is beyond that in which Goldman and Schauf (1973) report large differences between τ_c and τ_h in *Myxicola* axons.

In the presence of *n*PG, τ_c is reduced along with τ_h in axons in reversed [Na^+] gradient. However, in normal [Na^+] gradient τ_c remains close to control values even though τ_h has decreased. This suggests that at the holding potential the drug is removed from Na channels much more rapidly when the external Na^+ concentration is high. High $[Na^+]_0$ thus anatagonizes block of Na channels by *n*PG at high depolarizations and speeds recovery from block at negative potentials.

In a further test of this conclusion we examined two axons in normal [Na^+] gradients for "use-dependence" of block by *n*PG (Strichartz, 1973). A test pulse was applied and current recorded. A series of conditioning pulses was then generated, at a prescribed frequency, and was followed by a second test pulse. Voltages, pulse durations, and frequencies were chosen to keep the average membrane potential within 2 mV of the holding potential. In several cases conditioning pulses were composed of hyperpolarizing and depolarizing segments, with no resultant change in average V_m. Conditioning depolarizations were to +90 mV, a potential at which *n*PG is driven into the Na channel. At frequencies up to 10 Hz for up to 20-s control peak test currents were reduced by at most 8% and decay times not at all by the above conditioning protocol; these results were identical with *n*PG present as well. Thus, in this limited study, we found no evidence for use dependence of *n*PG action. This supports our earlier conclusion that in normal [Na^+] gradient *n*PG leaves the channel within 500 μs at −70 mV.

Structural Analogs

Arginine, the parent compound of *n*PG (Fig. 1) has been examined in a few experiments. Added internally at 5 mM to an axon in reversed [Na^+] gradient, arginine had no effect on decay time constants. Peak currents were blocked only at strong depolarizations. Block was zero below +42 mV and 38% at +112 mV. If inactivation was eliminated by internal perfusion with tannic acid (Shrager and Starkus, 1979) arginine again produced a relatively weak, voltage-dependent block, as did *n*PG. This has not as yet been studied in detail. It is perhaps not surprising that arginine is much weaker than *n*PG in blocking Na^+ channels. In contrast with the situation after incorporation into a peptide chain, the free amino acid contains charged amino and carboxyl groups in addition to the side chain guanidinium group.

Shortening the hydrocarbon chain of alkylguanidines results in compounds significantly less effective than *n*PG. Results for guanidinium (5 mM) and methylguanidinium (5 mM) are shown in Fig. 7 for both [Na^+] gradients (top, normal; bottom, reversed). Decay kinetics (left) and peak conductances (right) are affected only slightly in all cases. 5 mM *n*-propylammonium decreases τ and g_P by amounts about equal to those of 1 mM *n*PG (Lo and

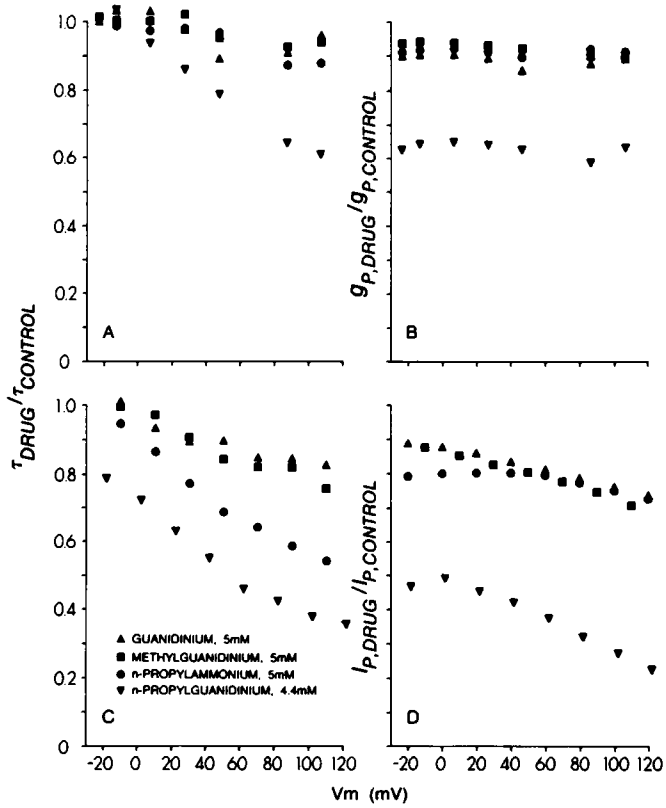

FIGURE 7 Analogs of *n*PG. Results are shown for axons in both normal (*A*, *B*) and reversed (*C*, *D*) [Na^+] gradients. Points give voltage dependence of ratios of decay time constant (*A*, *C*) and peak conductance (*B*) or current (*D*). Temperature = 8°C.

Shrager, 1981). The receptor for *n*PG thus seems capable of accepting the ammonium group, but with an affinity only one-fifth that of guanidinium.

DISCUSSION

The results presented set some limitations on possible models to explain the mechanism of action of *n*PG on sodium channels. In both [Na^+] gradients, the observed reduction in peak conductance was too great to be explained solely by the faster decay kinetics. Further, this was not due to a change in slow inactivation of sodium channels. This suggests a dual mechanism for *n*PG and we shall seek to find whether it is required to postulate two sites of interaction with the channel. We consider first whether *n*PG must enter the sodium channel in order to produce the effects noted. The decrease in both peak Na^+ conductance and in decay time was greater in reversed [Na^+] gradient than in normal [Na^+] gradient. High external Na^+ concentrations might antagonize *n*PG action if the drug's receptor were at the external surface of the membrane. However, our evidence suggests that this site is accessible only when nPG is applied internally. If the site is at the internal surface, and not inside the channel, then high internal [Na^+] might reduce the drug's effectiveness. This is not what we observed. If, however, *n*PG reaches its site from inside the axon, and yet is sensitive to high external [Na^+] then this interaction most likely takes place inside the sodium channel.

Further, the decreases in decay time constant in both [Na^+] gradients and the reduction in peak Na^+ conductance in reversed [Na^+] gradient are all strongly voltage dependent, with effects stronger at larger depolarizations. This also points to a site within the membrane. We conclude that *n*PG acts within the Na^+ channel. The small but significant change in reversal potential seen in normal [Na^+] gradient on addition of *n*PG suggests that *n*PG may alter the selectivity-determining region of the channel. Cahalan and Begenisich (1976) have noted that guanidinium alters sodium channel selectivity when perfused internally.

Our results thus far suggest that on depolarization the cationic *n*PG is driven into the sodium channel. The drug speeds decay kinetics and, in addition, blocks Na conductance. *n*PG is readily removed: displacement in normal [Na^+] gradient at -70 mV occurs within 500 μs, as shown in the double-pulse experiments. In order to characterize further the mechanism of interaction of *n*PG with the sodium channel new experiments were required. These results, along with a detailed kinetic model, are presented in the following paper (Lo and Shrager, 1981). We shall therefore reserve further discussion of the present observations until this work is described.

We thank Mrs. Wendy Keck for excellent secretarial assistance.

This work has been supported by the National Institutes of Health through research grant 5-R01-NS10500 and Research Career Development Award 1-K04-NS00133 (to Dr. Shrager).

Received for publication 30 July 1980 and in revised form 9 February 1981.

REFERENCES

Armstrong, C. M., and F. Bezanilla. 1977. Inactivation of the sodium channel. II. Gating current experiments. *J. Gen. Physiol.* 70:567–590.

Armstrong, C. M., F. Bezanilla, and E. Rojas. 1973. Destruction of sodium conductance inactivation in squid axons perfused with pronase. *J. Gen. Physiol.* 62:375–391.

Bean, B. 1979. Modification of sodium and potassium channel kinetics by diethyl ether and studies on sodium channel inactivation in the crayfish giant axon membrane. Thesis. University Microfilms, Ann Arbor, Mich.

Bezanilla, F., and C. M. Armstrong. 1977. Inactivation of the sodium channel. I. Sodium current experiments. *J. Gen. Physiol.* 70:549–566.

Cahalan, M. D., and W. Almers. 1979 *a*. Interaction between quaternary lidocaine, the sodium channel gates, and tetrodotoxin. *Biophys. J.* 27:39–56.

Cahalan, M. D., and W. Almers. 1979 *b*. Block of sodium conductance and gating current in squid giant axons poisoned with quaternary strychnine. *Biophys. J.* 27:57–74.

Cahalan, M., and T. Begenisich. 1976. Sodium channel selectivity. Dependence on internal permeant ion concentration. *J. Gen. Physiol.* 68:111–125.

Eaton, D. C., M. S. Brodwick, G. S. Oxford, and B. Rudy. 1978. Arginine-specific reagents remove sodium channel inactivation. *Nature (Lond.).* 271:473–475.

Goldman, L., and C. L. Schauf. 1973. Quantitative description of sodium and potassium currents and computed action potentials in *Myxicola* giant axons. *J. Gen. Physiol.* 61:361–384.

Hille, B. 1971. The permeability of the sodium channel to organic cations in myelinated nerve. *J. Gen. Physiol.* 58:599–619.

Hodgkin, A. L., and A. F. Huxley. 1952 *a*. The dual effect of membrane potential on sodium conductance in the giant axon of *Loligo*. *J. Physiol. (Lond.).* 116:497–506.

Hodgkin, A. L., and A. F. Huxley. 1952 *b*. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.).* 117:500–544.

Kirsch, G. E., J. Z. Yeh, J. M. Farley, and T. Narahashi. 1980. Alkylguanidine block of sodium channels in squid axon membrane. *Fed. Proc.* 39:380.

Lo, M-V. C., and P. Shrager. 1980. Sodium channel gating in the presence of arginine derivatives. *Fed. Proc.* 39:2063.

Lo, M-V. C., and P. Shrager. 1981. Block and inactivation of sodium channels in nerve by amino acid derivatives. II. Dependence on temperature and drug concentration. *Biophys. J.* 35:45–57.

Meves, H., and Y. Pichon. 1975. Effects of 4-aminopyridine on current in internally perfused giant axons of the squid. *J. Physiol. (Lond.).* 251:60.

Morello, R., W. Trzos, T. Begenisich, and J. K. Reed. 1980. Interaction of nonylguanidine with the sodium channel. *Biophys J.* 31:435–440.

Rojas, E., and B. Rudy. 1976. Destruction of the sodium conductance inactivation by a specific protease in perfused nerve fibers from *Loligo*. *J. Physiol. (Lond.).* 262:501–531.

Shrager, P. 1974. Ionic conductance changes in voltage clamped crayfish axons at low pH. *J. Gen. Physiol.* 64:666–690.

Shrager, P. 1977. Slow sodium inactivation in nerve after exposure to sulfhydryl blocking reagents. *J. Gen. Physiol.* 69:183–202.

Shrager, P., and J. G. Starkus. 1979. Block of sodium inactivation in nerve by polyphenols; ion accumulation in the Schwann cell space. *Biophys. J.* 25:306 *a*.

Starkus, J. G., and P. Shrager. 1978. Modification of slow sodium inactivation in nerve after internal perfusion with trypsin. *Am. J. Physiol* 235(5):C238–C244.

Strichartz, G. R. 1973. The inhibition of sodium currents in myelinated nerve by quaternary derivatives of lidocaine. *J. Gen. Physiol.* 62:37–57.

Van Harreveld, A. 1936. A physiologic solution for fresh water crustaceans. *Proc. Soc. Exp. Biol. Med.* 34:428–432.

Yeh, J. Z., and C. M. Armstrong. 1978. Immobilization of gating charge by a substance that simulates inactivation. *Nature (Lond.).* 273:387–389.

Yeh, J. Z., G. S. Oxford, C. H. Wu, and T. Narahashi. 1976. Interactions of aminopyridines with potassium channels of squid axon membranes. *Biophys. J.* 16:77–81.

Yeh, J. Z., and T. Narahashi. 1977. Kinetic analysis of pancuronium interaction with sodium channels in squid axon membranes. *J. Gen. Physiol.* 69:293–323.