

Aminopyridine Block of Transient Potassium Current

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ABSTRACT The blocking action of 4-aminopyridine (4-AP) and 3,4-diaminopyridine (di-AP) on transient potassium current (I_A) in molluscan central neurons was studied in internal perfusion voltage-clamp experiments. Identical blocking effects were seen when the drugs were applied either externally or internally. It was found that aminopyridines have two kinds of effects on I_A channels. The first involves block of open channels during depolarizing pulses and results in a shortening of the time to peak current and an increase in the initial rate of decay of current. This effect of the drug is similar to the block of delayed potassium current by tetraethylammonium (TEA). The other effect is a steady block that increases in strength during hyperpolarization, is removed by depolarization, and is dependent on the frequency of stimulation. The voltage dependence of steady state block approximates the voltage dependence of inactivation gating and changes e-fold in ~ 10 mV. These data suggest that the strength of block may depend on the state of I_A gating such that the resting state of the channel with open inactivation gate is more susceptible to block than are the open or inactivated states. A multistate sequential model for I_A gating and voltage-dependent AP block is developed.

INTRODUCTION

Potassium currents in nerve and muscle are susceptible to block by aminopyridines (Pelhate and Pichon, 1974; Ulbricht and Wagner, 1976; Yeh et al., 1976*a, b*; Meves and Pichon, 1977). These drugs are of interest as pharmacological tools for the suppression and separation of K^+ currents. Their physiological effects include increase in the release of transmitter at the neuromuscular junction and production of epileptiform discharge when applied to mammalian brain, effects that may result from block of potassium channels. In molluscan central neurons, the fast transient potassium current I_A (for review see Adams et al., 1980) is particularly sensitive to block by aminopyridines (Thompson, 1977; Adams and Gage, 1979). The blocking action is dependent on voltage, frequency of stimulation, and prior activation of channels in a manner that differs from the aminopyridine block of delayed outward current in nerve.

In this paper the mechanism of aminopyridine block of I_A is considered. It is concluded that block involves two kinds of effects. The time to peak current during a depolarization is shortened in the presence of AP and the initial rate

of fall of current is hastened, which indicates a blocking effect involving open I_A channels. In addition, there is a steady state blocking action that increases in strength during hyperpolarizing pulses that remove inactivation of I_A . Steady state block is decreased by depolarization. The rate of inactivation of blocked channels is modified in a way that suggests an interaction between the inactivation mechanism and the drug, and it is suggested that the inactivation mechanism is essential for mediating the frequency dependence and voltage dependence of drug action. These findings lead to the development of a model for AP block based on the hypothesis that the strength of block depends on the state of I_A gating.

METHODS

Preparation

Experiments were performed on neuron somata taken from the pleural ganglia of the nudibranchs *Archidoris* and *Anisodoris*. Ganglia were removed and treated for 2 min with pronase to soften the outer muscle and connective tissue sheaths. Epineural sheaths were dissected away and small clumps of cells were axotomized and removed using the method of Connor (1977). The cells were transferred to a recording chamber and cooled to 10°C.

Voltage Clamp

An internal perfusion voltage clamp was used that employs a suction pipette as a cannula for internal perfusion and as a current electrode. A separate 3 M KCl microelectrode (2–5 M Ω) is used to measure cell voltage. Similar techniques have been described by Kostyuk et al. (1975), Lee et al. (1978), and Takahashi and Yoshii (1978). The suction pipette is a 3-mm-Diam glass capillary that is pulled to a point and then cleaved and fire-polished to form a 40- μ m-Diam orifice. The pipette is fitted with a thinner internal glass capillary that has a tip diameter of 20 μ m and is manipulated to within 100 μ m of the suction pipette tip. Internal solutions are supplied to the cell through this inner capillary and exit out the back of the suction pipette. The flow of internal solution and the negative pressure developed at the pipette tip are produced by vacuum and controlled by a needle valve to maintain a flow rate of 0.2–0.5 ml/min. The electrical resistance of the suction pipette is 350 k Ω in saline. When it is brought up to contact a cell and negative pressure is applied, this resistance increases 10–20 times. As the cell membrane in the pipette orifice ruptures, the resistance drops again to a value of 2–5 M Ω when measured at voltages close to the cell resting potential (–40 mV). This value is similar to the membrane resistance measured in the same cells with a two-microelectrode voltage clamp. Spike waveform and amplitude and resting potential (after correction for liquid junction potentials), measured by the perfusion pipette, are the same as simultaneous measurements with a microelectrode. Evidently a tight seal is formed between the glass pipette and the cell. The perfusion pipette is acid cleaned with Chromerge (Fisher Scientific Co., Pittsburgh, PA) after each experiment. The cleaning treatment increases the value of the initial resistance measured before cell puncture by a factor of two to five.

After the initial rupture of the membrane at the orifice of the perfusion pipette, the perfusate was routinely switched to one containing papain (1 mg/ml) for 10–15 min. This enzyme treatment has the effect of decreasing the time needed to change internal ion concentrations. It is not an essential procedure, however, and in control experi-

ments it was shown to have no measurable effect on the parameters reported in this study. The adequacy of perfusion was checked by switching to internal solutions without K⁺ (solutions containing Cs-glutamate or Tris-glutamate in place of K-aspartate) and monitoring the amplitude of membrane potassium currents and the potassium reversal potential with standard voltage-clamp procedures. A 90% reduction of I_A and I_K is routinely achieved in 20 min. The effect is reversible.

The compositions of saline solutions used to bath the cells and for internal perfusion of cells are shown in Table I. 4-aminopyridine (4-AP) and 3,4-diaminopyridine (di-AP) were obtained from Aldrich Chemical Co., Milwaukee, WI. 3-aminopyridine (3-AP) was obtained from Sigma Chemical Co., St. Louis, MO. The drugs were added fresh to the salines just before use and before final pH adjustment. Data were recorded on film, chart paper, and FM magnetic tape, and analyzed either by hand or digitized and analyzed with a laboratory microcomputer.

RESULTS

Transient potassium current, I_A , activates with a delay upon depolarization, reaches a peak in 30–50 ms, and then inactivates over an exponential time course. The voltage dependence of I_A is centered about the resting potential of -40 mV. Inactivation is nearly complete at that voltage and is removed

TABLE I
SALINE SOLUTIONS

<i>mM</i>	Na	K	Ca	Mg	Cl	Sucrose	Hepes	pH	EGTA
External	460	10	10	50	590	—	10	8.0	0
Internal	50	375 (aspartate)	—	5	16	300	20	7.6	1

over an exponential time course by a conditioning hyperpolarization. Complete removal of inactivation normally occurs below -100 mV. Removal of inactivation by hyperpolarizing conditioning pulses is shown in Fig. 1B. Activation of I_A increases with the size of depolarizing steps from a negative holding voltage and first appears above -50 mV, as seen in Fig. 1A. Because activation begins near the resting potential, there is a voltage range between -50 and about -30 mV where I_A can be studied in isolation, free of significant contamination by other membrane currents (see Adams et al., 1980). At more positive voltages, Na⁺, Ca⁺⁺, and other K⁺ currents begin to activate and contaminate the records of I_A . Because the effects of aminopyridines on these other currents are not known in detail, this study is mainly restricted to the voltage range between -100 and about -30 mV.

The time course and voltage dependence of normal I_A gating have been described by Connor and Stevens (1971) and by Neher (1971). Curves showing the steady state voltage dependence of activation and of inactivation are shown in Fig. 1C. The kinetics of both the activation and inactivation reactions are not strongly dependent on voltage in the range studied here. At -30 mV the activation time constant in different cells was 6–8 ms and the inactivation time constant was 150–410 ms.

External and Internal AP Blocks I_A

The effect of external application of 3 mM 4-aminopyridine is illustrated in Fig. 2, which shows a control current and subsequent test currents recorded at 5-min intervals after adding the drug to the bath. The drug caused a 60% reduction in I_A amplitude in this experiment, which developed over a 20-min period. 3,4-diaminopyridine appears to be a more powerful blocking agent

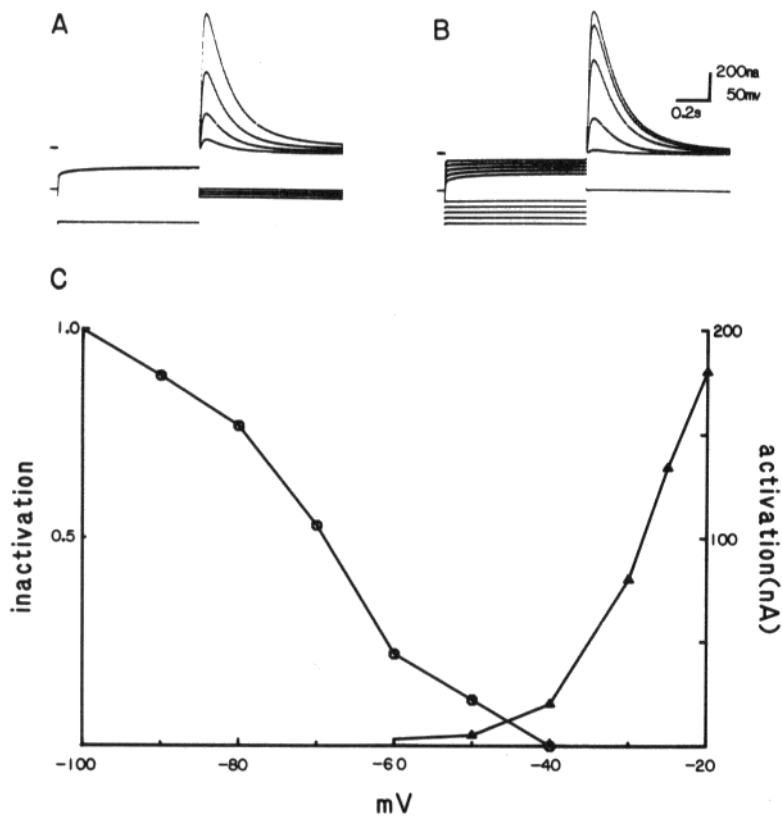


FIGURE 1. Activation and inactivation gating of I_A in normal external and internal solutions. (A) Increasing activation with increasing depolarization. A prepulse to -100 mV removes inactivation. (B) Hyperpolarizing conditioning pulses remove inactivation. The cell was held at -40 mV, stepped to a series of voltages between -100 and -50 mV and then stepped to -30 mV to activate I_A . (C) Voltage dependence of inactivation and activation.

and produces $>90\%$ reduction in amplitude at this concentration when tested by the same protocol. A consistent feature of the effect of both drugs is a dramatic change in the current waveform. The current does not inactivate exponentially in the presence of aminopyridines (AP) but shows an initial phase that has a time course somewhat faster than normal inactivation followed by a later, very slow decline in current. These changes in time course

develop as the drug takes effect and lead to crossovers in the current traces. Because of the slow decline in current, the decay of I_A lasts 8–10 times longer than normal. The slow decline becomes more prominent with increased drug concentration when repetitive depolarizations are applied, or when the duration of a conditioning hyperpolarization is increased. The changes in current waveform suggest some interference by AP with the rate of inactivation at depolarized voltages. Evidence presented below indicates that the slow phase of current decay may result from slow, voltage-dependent unblocking of channels during depolarization followed by inactivation of these newly unblocked channels.

It was found that 4-aminopyridine (4-AP) and 3,4-diaminopyridine (di-AP) each had the same effects whether applied externally or internally. Both

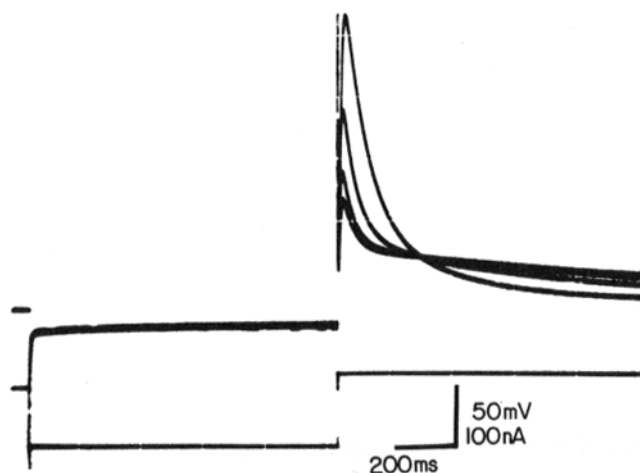


FIGURE 2. Increasing block of I_A as 3 mM external 4-AP takes effect. A control pulse and subsequent test pulses applied 5, 10, 15, and 20 min after application of the drug are shown. Note crossovers in time course of inactivation. Holding voltage is -40 mV; conditioning pulse to -100 mV for 1 s; test voltage is -30 mV. Normal internal solution.

compounds are monovalent cations at the pH used in this study (Albert, 1963). 3-aminopyridine, which is uncharged at neutral pH, is a much less potent blocker and causes only 15% block at a concentration of 5 mM applied either externally or internally. 3-AP was not studied further.

In experiments that use less than saturating doses of blocker, some fraction of I_A channels remain unblocked at the beginning of test depolarizations. Current in unblocked channels apparently accounts for the early peak I_A in Fig. 2 because the peak is reduced by higher concentrations of blocker. Aminopyridines appear to have an effect on these unblocked channels. The time to peak current is decreased and the initial time course of current decay is speeded in the presence of AP (see Fig. 2). These results could be explained if AP has a TEA-like action on open channels, interacting with the channels

to block current. By measuring the rate of decay of I_A transients early during depolarizations, it was determined that in 3 mM external 4-AP the apparent time constant of inactivation was about two-thirds as large as the control value. This could in itself account for as much as 15% of the observed block of I_A . It is not surprising that, as organic amine cations, aminopyridines would have this kind of effect for which there are many precedents in the literature (for example see Armstrong, 1969). This blocking action is difficult to study, however, because of the very limited voltage range over which uncontaminated I_A can be recorded and because of the complication of a later prolonged decay of current. The late slow decline in I_A differs from the effect on initially unblocked channels and because of its slow time course is easily separable from it.

Block Increases during Hyperpolarization

A consistent feature of the effect of AP is that the strength of block depends on the protocol used to remove I_A inactivation. This is illustrated in Fig. 3

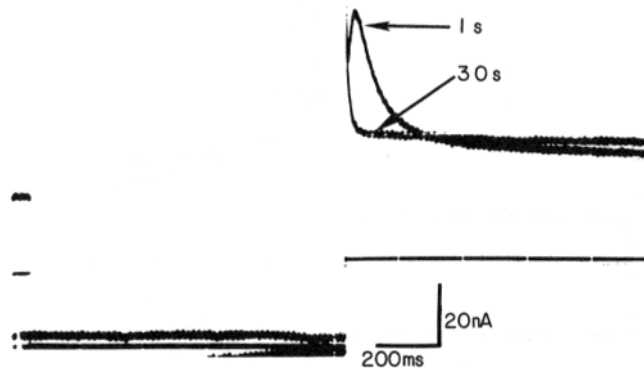


FIGURE 3. Hyperpolarization increases block. In the trace labeled "1 s" the cell was held at -40 mV and stepped to -100 mV for 1 s before a test depolarization to -30 mV. In the other trace (labeled "30 s") the cell was held at -100 mV for 30 s before the test step to -30 mV. External solution contained 1.5 mM di-AP; normal internal solution. The rapid relaxation in the trace labeled "30 s" is an artifact of the 1-kHz low-pass filter. Note crossover in current traces during the falling phase of current.

where two current records taken from a cell bathed in 1.5 mM di-AP are superimposed. In one of the traces (labeled "1 s") the cell was held at -40 mV, stepped to -100 mV for 1 s, and returned to -30 mV in order to activate I_A . In the other trace (labeled "30 s") the cell was conditioned at -100 mV for 30 s before the step to -30 mV, with the result that the strength of block is greatly increased at early times and somewhat lessened at late times. In a normal saline solution without AP, these two traces would have superimposed.

The time course of this effect of conditioning hyperpolarization is shown in Fig. 4, where the duration of a hyperpolarizing conditioning step to -100 mV was progressively increased while the cell was internally perfused with 1 mM di-AP. As the conditioning step was lengthened, the peak current first in-

creased and then decreased (Fig. 4B). This is not seen in the absence of drug (Fig. 4A), where the peak current simply increases toward a saturating amplitude as inactivation is progressively removed by longer conditioning steps. The drug caused a 65% block of I_A after a 2.5-s conditioning hyperpolarization but only a 23% block after a 250-ms conditioning pulse. In this experiment it appears that there are two simultaneous processes governing the

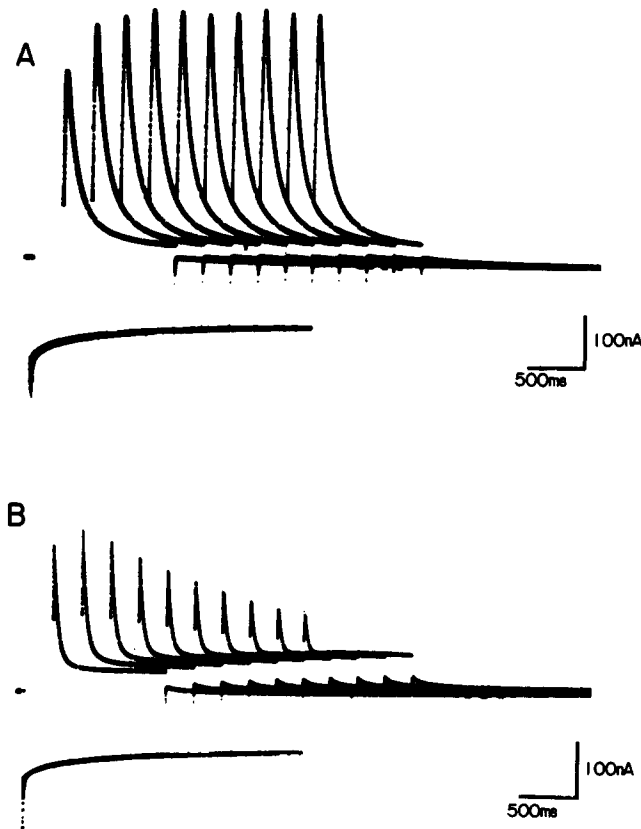


FIGURE 4. Time course of block during conditioning hyperpolarization. (A) Control record. The cell was held at -40 mV and stepped to -100 mV for intervals between 250 and 2,500 ms with the interval changing in 250-ms increments. I_A is seen on depolarizing to -30 mV after each conditioning pulse. 30 s separated each trial. Normal internal and external solutions. (B) Repeat of the experiment while the cell was internally perfused with saline containing 1 mM di-AP.

number of I_A channels available for activation by depolarization. The normal process of removal of inactivation during a hyperpolarizing conditioning pulse is superimposed upon a progressive, slower increase in the number of blocked channels. From the control record (Fig. 4A), it was determined that the time constant of removal of inactivation at -100 mV in this cell is 319 ms. The progressive increase in the number of blocked channels in Fig. 4B occurs

exponentially but much more slowly and can be described by a time constant of 2.4 s. This slow time constant presumably reflects the rate of interaction of AP with some site on or near the channel at this voltage.

The fact that block increases with hyperpolarizing conditioning pulse raises the possibility that the strength of block may be dependent in some way on the process of inactivation gating. The voltage dependence of block by AP was measured in a three-pulse experiment and is compared with the voltage dependence of normal inactivation in Fig. 5. The voltage command program

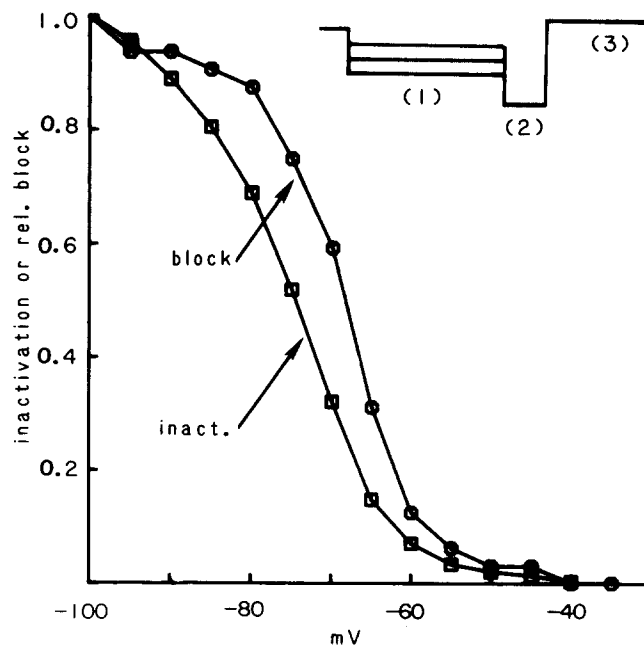


FIGURE 5. Comparison of voltage dependence of block with the voltage dependence of normal inactivation. The curve labeled "block" shows the strength of block as a function of conditioning voltage and was calculated using the method described in the text and the pulse paradigm shown in the figure insert. The cell was exposed to 1 mM diAP externally; normal internal saline. The curve labeled "inact." shows the voltage dependence of inactivation for the same cell in normal internal and external salines.

used to measure the voltage dependence of block is shown in the figure insert. The cell was bathed in 2 mM external di-aminopyridine and held at -40 mV for 30 s between trials. Voltage was stepped to a series of different conditioning voltages (pulse 1) for 10 s, followed by a step to -100 mV for 1 s (pulse 2). The purpose of this second pulse is to allow complete removal of inactivation in unblocked channels. A depolarization to -30 mV was used as a test step (pulse 3). The peak amplitude of I_A seen during the test step gives a measure of the level of block achieved during the conditioning pulse (pulse 1). Peak currents after each conditioning pulse were normalized by dividing by the

current observed with a pulse-1 conditioning voltage of -30 mV. The curve labeled "block" in Fig. 5 was then constructed by plotting one minus the normalized peak current against the voltage during pulse 1. It is a curve of the strength of block at different conditioning voltages in 2 mM di-AP. The other curve shown in Fig. 5 (labeled "inact.") is the voltage dependence of I_A inactivation measured in the same cell before AP was applied. The points in this curve are the peak amplitudes of I_A observed on stepping to -30 mV from a series of different 1-s-long conditioning voltages. Normalized peak current was plotted against conditioning voltage to construct the curve. These experiments were repeated eight times with consistent results. The data shown are from one cell in this series.

It is apparent from Fig. 5 that the voltage dependence of block covers the same voltage range as normal inactivation gating and that the two curves have approximately the same shape. The curve depicting the voltage dependence of block lies ~ 5 mV toward the right of the curve for inactivation. Also, the voltage dependence of block appears to be somewhat steeper than that of inactivation.

The similarities between the two curves in Fig. 5 suggest that the effectiveness of AP as a blocker may be dependent on the state of inactivation gating. Alternatively, the blocking action might be simply voltage dependent and insensitive to inactivation gating. For the second alternative to occur, it is generally postulated that the free energy of binding of the charged blocking molecule to a site in the channel is altered electrostatically by the membrane voltage field. Although this kind of action is not ruled out by these experiments, it is noted that the steepness of the voltage dependence of AP block (9.5 mV for e-fold change in blocking strength) is greater than the maximum of 25 mV for e-fold change expected for a monovalent blocking molecule responding only to the membrane voltage field. It is concluded, therefore, that the steep voltage dependence of block does not result simply from electrophoresis of AP to a site within the membrane, and it is suggested instead that access to the blocking site or formation of the site depends on the open state of inactivation gating.

Frequency Dependence

The fraction of channels blocked by AP is modified by applying repetitive test steps and is sensitive to the frequency of repetition. To produce the records shown in Fig. 6, voltage-clamp steps were applied at a rate of 1/s both in normal saline (Fig. 6A) and during internal perfusion with 2 mM 4-AP (Fig. 6B). The cell voltage was held at -40 mV, and stepped to -100 mV for 1 s to remove inactivation. The voltage was then stepped to -30 mV to activate I_A . With repetitive clamp steps in AP there is a progressive decrease in peak outward current. Also, the time course of current decay changes progressively due to an increase in the prolonged phase of current decay that leads to crossovers in the current traces. The changes in current during repetitive activation mirror the changes seen over time as the drug takes effect as shown in Fig. 2. These effects are not seen in the absence of AP (Fig. 6A). Both the incremental

increase in the number of blocked channels and the fraction of channels blocked in the steady state increase at higher rates or repetition. Frequency dependence is seen whether the drug is applied internally or externally and is more pronounced with the stronger blocker, di-AP.

One explanation for frequency-dependent block is that the fraction of channels that are blocked increases during the 1-s hyperpolarizing condition-

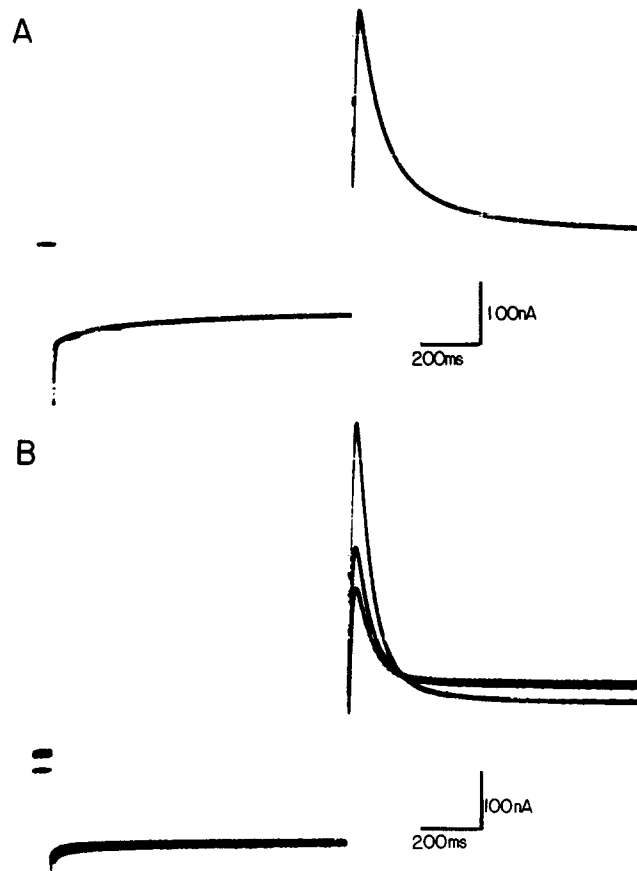


FIGURE 6. Frequency-dependent block by AP. (A) Control record. The cell was held at -40 mV and a pulse sequence consisting of a prepulse to -100 mV for 1 s and a test pulse to -30 mV for 1 s was repeated five times with a 1-s interval between pulses. Normal internal and external salines. (B) The experiment was repeated on a different cell, which was internally perfused with 2 mM 4-AP. Normal external saline.

ing step used to open inactivation gates. This process proceeds slowly (Fig. 4B) so that during repetitive clamp steps using the protocol of Fig. 6, an increasing number of channels may be blocked during each successive conditioning hyperpolarization, resulting in a reduction in I_A amplitude when the membrane is subsequently depolarized.

Recovery from Block on Depolarization

From the record shown in Fig. 3, it is apparent that a significant recovery from block occurs at the holding potential of -40 mV relative to the level of block at -100 mV. Other evidence for removal of block by depolarization is shown in Fig. 7. Repetitive depolarization at 1-s intervals from a holding potential of -100 mV to a test potential of -30 mV results in a progressive increase in peak I_A when AP is present. In normal saline there is no change in current amplitude with repetition at this frequency. Apparently some percentage of blocked channels becomes unblocked during the pulses to -30 mV. Unblocking of I_A channels during a single depolarization is shown in Fig. 8. The cell was bathed in 2 mM external di-AP and held at -100 mV for 30 s

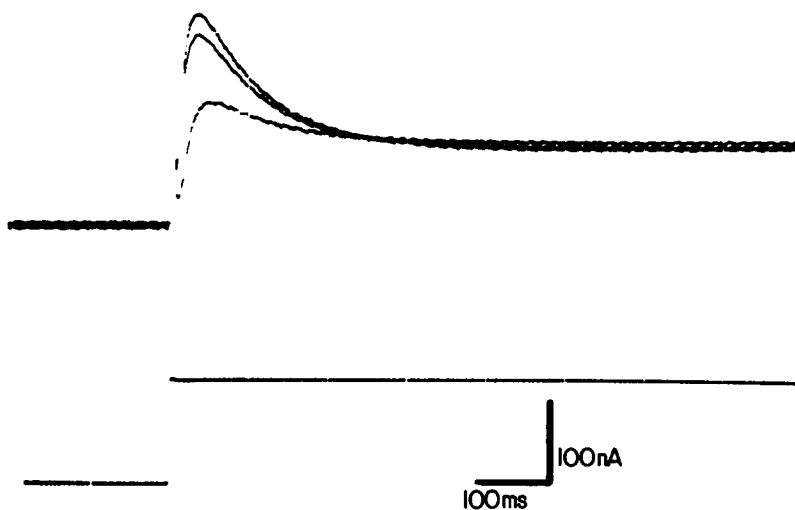


FIGURE 7. Frequency-dependent unblocking. The cell was held at -100 mV and pulsed three times to -30 mV for 1 s with a 1-s interval between pulses. The external solution contained 1 mM di-AP; the internal solution was normal. The peak outward current increases during the three trials. With continued repetition this effect saturates.

before a depolarization to -30 mV. Just after the depolarizing step there is a rapid outward current transient that results from activation of initially unblocked channels. After the initial I_A transient there is a secondary slow growth of current followed by a very prolonged current decay that could result from the unblocking of I_A channels followed by their inactivation.

The kinetics of the unblocking reaction were studied in a three-pulse experiment (not shown) where the duration of a conditioning depolarization (pulse 1) was changed and the percentage of unblocked channels was assessed from the peak amplitude of I_A during a later test pulse to -30 mV (pulse 3). An intermediate pulse to -100 mV for 1 s (pulse 2) was applied to remove inactivation from unblocked channels. The results of this experiment indicated that block is removed over an exponential time course with a time constant of

between 1.5 and 4.0 s in different cells. The value of this time constant is not dependent on voltage when measured between -60 and -20 mV. It is apparent that the unblocking reaction is very slow compared with the kinetics of normal gating. At the time of peak I_A , ~ 30 ms, unblocking would have proceeded to a very small extent and would not perceptibly disturb the amplitude. The current caused by unblocking only becomes apparent later, after the I_A transient and after current in normal channels had inactivated significantly.

These observations on the rate of unblocking, combined with the fact that inactivation, which is a much faster process, is normally complete (i.e., $h = 0$) above -40 mV, lead to a paradox. If unblocking is slow compared with inactivation, and if inactivation goes to completion at the test voltage, why is a redevelopment of current seen caused by the unblocking of channels on

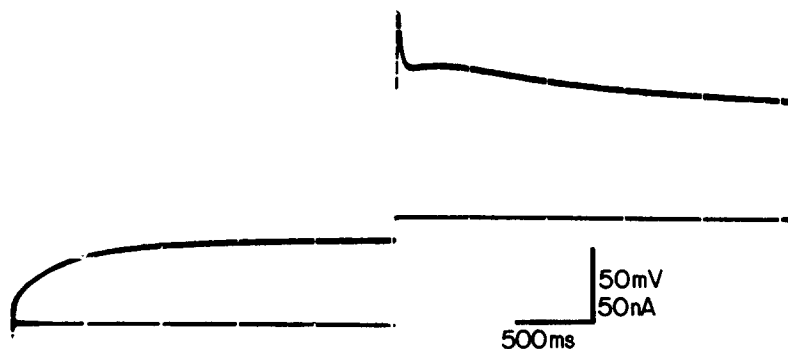


FIGURE 8. Unblocking during a single pulse. The cell was bathed in 2 mM di-AP. Holding voltage was -40 mV. A conditioning step to -100 mV for 1 s preceded a test step to -30 mV. A slow recovery and decay of I_A is seen after the transient current caused by activation and inactivation of unblocked channels is over. Normal internal solution.

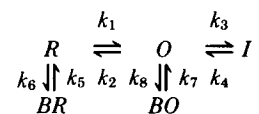
depolarization? This might be explained, and the reappearance of current understood, if blocked channels do not inactivate and if inactivation can only proceed after unblocking and opening of channels. Such an effect would be seen if the binding of AP to the channel shifted the voltage dependence of inactivation toward more depolarized voltages or if by some other mechanism the blocking ion prevents the closing of inactivation gates. This explanation is supported by the observation that the rate of recovery from block measured at a given voltage in a three-pulse experiment (for example, 1.7 s at -30 mV), is very similar to the final slow rate of decay of I_A during a single depolarization to the same voltage (1.5 s in 3 mM external 4-AP). It appears that the rate-limiting step governing the slow decay of current is the unblocking rate rather than the rates of activation or inactivation of newly unblocked channels. The slow fall of current during depolarization indicates that AP interferes with the

normal process of inactivation, preventing it from occurring when AP is at its blocking site.

Multistate Model for Frequency-dependent Block

A multistate sequential model for I_A channel gating and channel drug interactions was developed that simulates the voltage-dependent and frequency-dependent blocking action of AP. The model is based on that proposed by Yeh et al. (1976a) for AP block of delayed outward current in squid axons. It differs from their model by the addition of an inactivation step, by the stipulation that AP interferes with the process of inactivation, and by including AP block of open channels during depolarization.

The model for I_A gating and AP blocking reactions is summarized in this reaction scheme:



The channel is thought to exist normally in one of three states: a resting state (R), an open state (O), and an inactivated state (I). It is assumed that transitions between states occur according to a linear reaction sequence and that the kinetics of channel gating arise from the voltage dependence of the rate constants for the various transitions. This reaction sequence is a simplification because it does not predict the characteristic delay in activation of I_A during depolarization. This can be corrected by adding three or four nonconducting states to the left of R (for example see Yeh et al., 1976a; Armstrong and Bezanilla, 1977), but this elaboration is not necessary for the present purpose of modeling the block by AP.

Aminopyridines are thought to act by binding a site or sites at the channel with the result that current in the channel is blocked. It is assumed that channels in the resting state with open inactivation gates are susceptible to block and can enter a blocked state (BR). This blocking reaction shows a voltage dependence similar to the removal of inactivation as shown in Fig. 3. In addition, open channels are susceptible to block caused by a more rapid interaction with AP during depolarization as shown by the transition from O to BO .

Blocked channels may occupy either resting or open states but experimental evidence suggests that blocked channels are prevented from inactivating. It is assumed that recovery occurs during depolarization because the transition from the blocked resting state (BR) to the resting state (R) is favored at depolarized voltages. The two blocked states (BR and BO) are treated separately. This is equivalent to the assumption that there are two independent binding sites for AP.

An attempt was made to use experimentally measured rate constants in the model, and for this purpose an exemplar cell was chosen for which a reasonably

complete set of data existed. The exemplar cell was the same as that shown in Fig. 2. In general, the rate constants used in the model were derived from measured time constants using the relationship

$$\tau = \frac{1}{k_a + k_b},$$

where τ is the time constant and k_a and k_b are forward and backward rate constants. In simulating channel gating and the transitions between the blocked state BR and the resting state R during depolarization, the backward rate constant for each transition was set to zero and the forward rate constant was taken to be the reciprocal of the measured time constant. Just the opposite was done during hyperpolarization. This is a simplification because the forward and backward rate constants would in reality be continuous variables that depend on voltage. This approach was adopted because there is insufficient data available on the mechanism of I_A gating and on the voltage dependence of individual rate constants to justify a more thorough treatment. The chosen rate constants are shown in Table II.

TABLE II
RATE CONSTANTS FOR BLOCKING MODEL

	Rate constant		Source
	-40 mV	-100 mV	
	s^{-1}		
k_1	100	0	Connor and Stevens (1971)
k_2	0	100	
k_3	6.7	0	Fig. 1
k_4	0	6.7	
k_5	0.63	0	Results
k_6	0	0.63	
k_7	3.4	3.4	Results
k_8	3.4	3.4	

For the block of open channels, the forward and backward rate constants were assigned in the following way. The initial rate of fall of the I_A transient was measured in the presence of 3 mM external 4-AP and compared with the rate of inactivation of channels measured without blocker. It was found that the initial fall is 1.5 times faster in AP ($\tau = 78$ ms compared with 150 ms). This means that if the blocking reaction follows an exponential time course it must proceed with a time constant of 100 ms. The strength of the block of open channels is not known in detail, and because of this the ratio of forward to backward rate constants for this reaction was varied systematically in order to achieve a reasonable fit to the data (Table II).

Solutions of the model were calculated using the point-slope method implemented on a PDP 1103 microcomputer. Conductance waveforms were calculated from the assumption that conductance is proportional to the number of open channels, $o(v,t)$.

Two solutions of the model are shown in Fig. 9. Panel A shows the change in conductance time course with exposure to AP and should be compared with the experimental results shown in Fig. 2. For this example, a holding voltage of -100 mV was assigned so that all channels would be in either the resting state *R* or the blocked state *BR*. The larger trace shows the time course of conductance in the absence of blocker, whereas the smaller trace shows the time course when the steady state blocking level is 50%. Panel B shows frequency-dependent block during three repetitions of a voltage-clamp program wherein the cell was held at -40 mV, stepped to -100 mV for 1 s, and then returned to -40 mV. This pulse schedule was repeated at 1 Hz. The initial level of steady state block was 30%. This record should be compared with the experimental data shown in Fig. 6B. During repetitive hyperpolarizations in the model, blocked channels accumulate because hyperpolarization favors the blocking reaction and because recovery from block proceeds slowly and is incomplete during the interpulse interval.

The model presented here provides a plausible mechanism for AP block and will reproduce several features of the data. These include frequency-dependent and voltage-dependent block, crossovers during the falling phase of current, prolongation of the final return to baseline during depolarization, shortening of the time to peak, and hastening of the initial fall of the I_A transient. Quantitatively the model fails at several points. The time at which blocked and unblocked currents cross is too late in the model and, therefore, the crossover occurs at too low a current level. Also the decrease in time to peak is not as prominent as that seen in experiments where di-AP was the blocking ion.

DISCUSSION

Pelhate and Pichon (1974) first found that AP blocks the outward current in cockroach axons. Yeh et al. (1976*a, b*) studied the kinetics of block of delayed outward current in squid axon in detail. Several comparisons can be drawn between the action of aminopyridines on delayed outward currents and their action on I_A . In both cases block is removed by strong depolarization, by longer depolarization, and by increased frequency of depolarization. These results were interpreted in terms of a model in which AP molecules are thought to bind to closed K^+ channels and to be released from open K^+ channels in a voltage-dependent manner (Yeh et al., 1976*a*). The unblocking reaction is slower than normal gating, as is the re-establishment of block on hyperpolarization, and therefore one sees use-dependent recovery from block during repetitive depolarization in squid axon delayed outward current as well as in I_A channels. Recovery from block upon depolarization has also been reported for voltage-dependent delayed outward current at the node of Ranvier (Ulbricht and Wagner, 1976) and in molluscan nerve cell bodies (Herman and Gorman, 1981). There are additional effects of AP on I_A that are not shared by delayed outward currents in axons. These additional effects appear to involve the inactivation mechanism.

In I_A channels the voltage dependence of block follows to a close approxi-

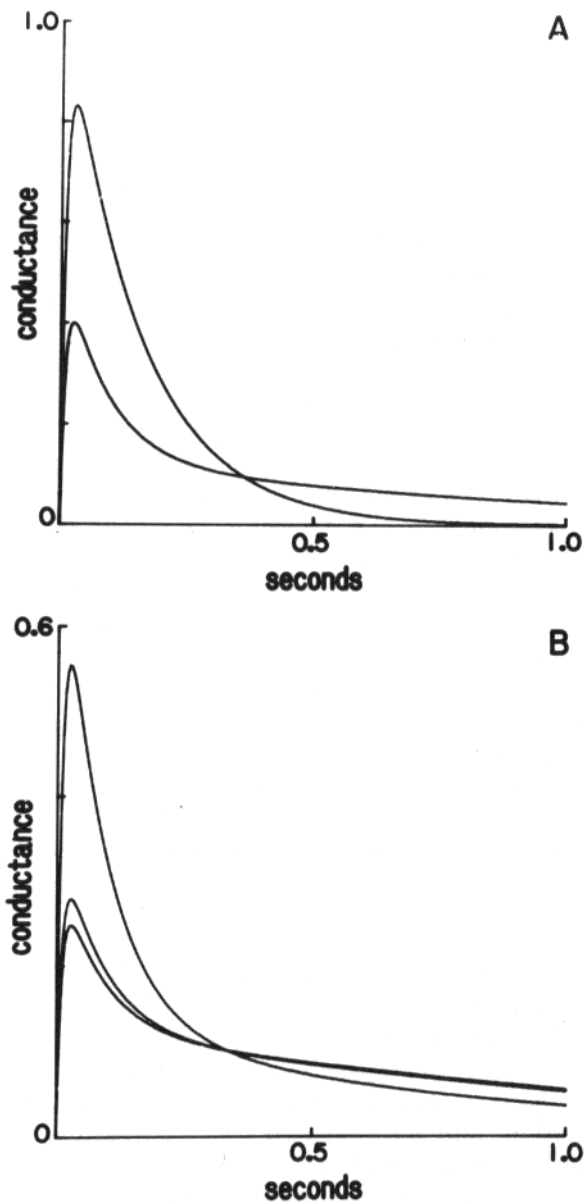


FIGURE 9. Simulation of AP block using the sequential model. The rate constants used in these calculations are given in Table II. (A) Change in conductance time course on exposure to blocker. The larger trace is a control corresponding to a cell held at -100 mV and stepped to -30 mV in normal saline. The smaller trace simulates the effects of exposure to AP. The initial occupancies of states used in these calculations were: (1) unblocked; $R = 100\%$; all other states equal 0% ; and (2) blocked; $R = 50\%$; $BR = 50\%$; all other states equal 0% . (B) Frequency-dependent block. The voltage was initially -40 mV so that inactivation could be assumed to be complete. An initial steady state block of 30% was assigned. Three repetitive hyperpolarizing pulses to -100 mV for 1 s were applied and the time course of conductance on stepping back to -40 mV is shown. Hyperpolarizations were repeated at a rate of 1 Hz. The percentage of channels blocked just before each depolarization was: (1) 30% ; (2) 65% ; (3) 69.5% .

mation the voltage dependence of normal inactivation gating. One interpretation of these data is that the strength of AP block is modulated by inactivation either because in the inactivated state access of AP to a receptor site in the channel is prevented or because the formation of a receptor for AP depends on the open state of inactivation gates. This may be viewed as a competition between the normal inactivation process and AP block. The two processes have identical effects, i.e., they block current and are separable in these experiments only because the kinetics of AP reactions with the channel are so much slower than channel gating.

Aminopyridines interfere directly with the process of inactivation. The pronounced slow phase of current during depolarization in AP could only occur if inactivation gating of blocked channels were prevented in some way. This might be explained if in the presence of drug, blocked channels cannot proceed to an inactive state, but as unblocking slowly occurs during long depolarizations or incrementally during repeated depolarizations, newly unblocked and conducting channels can proceed with inactivation. This is interpreted as evidence for the sequential model for I_A gating that was presented in Results. It is not yet clear how AP prevents inactivation, but it may act by shifting the voltage dependence of inactivation gating toward more depolarized voltages so that channels with bound AP do not inactivate at voltages where inactivation normally goes to completion. Alternatively, when AP interacts with the channel it may prevent inactivation gating by steric interactions. These effects bear a similarity to the action of *N*-methylstrychnine and pancuronium ions on sodium channels. Both of these compounds evidently compete with the normal inactivation mechanism preventing inactivation while blocking the channel (Cahalan et al., 1980).

Aminopyridines provide useful pharmacological tools for blocking I_A . The strong voltage dependence of blocking action means, however, that some care must be used in interpreting the results of experiments wherein AP is used to pharmacologically separate I_A from other membrane currents so that the activation of these other currents can be measured. Since the block is largely removed at voltages near the resting potential in these molluscan cell bodies, test pulses would have to be preceded by a sufficiently deep and long conditioning hyperpolarization in order to ensure adequate suppression of I_A . To verify the separation of I_A from other membrane currents it is not adequate to test for block of I_A with a single protocol. Instead, it will be necessary to show that the block is effective for the voltage command programs and repetition frequencies used in experiments.

I would like especially to thank R. Aldrich and B. Jones for help with computer modeling, Sharon Smith for help in the construction of the perfusion apparatus, and J. Yeh for helpful suggestions. I am also grateful to R. Aldrich, C. Scheffey, and W. F. Gilly for their critical reading of the manuscript, to B. Dyer for preparation of figures, and to Sharon Nugent for secretarial help.

This work was supported by National Institutes of Health grant NS 14519.

Received for publication 10 August 1981 and in revised form 1 December 1981.

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