Differential Blockage of Two Types of Potassium Channels in the Crab Giant Axon

Bernat Soria, Nelson Arispe, M. Emilia Quinta-Ferreira, and Eduardo Rojas

Department of Biophysics, School of Biological Sciences, University of East Anglia, Norwich, England, and Laboratory of Cell Biology and Genetics, NIADDK, NIH, Bethesda, Maryland 20205

Summary. Measurements were made of the kinetic and steady-state characteristics of the potassium conductance in the giant axon of the crabs *Carcinus maenas* and *Cancer pagirus*. The conductance increase during depolarizing voltage-clamp pulses was analyzed assuming that two separate types of potassium channels exist in these axons (M.E. Quinta-Ferreira, E. Rojas and N. Arispe, *J. Membrane Biol.* **66:**171–181, 1982). It is shown here that, with small concentrations of conventional K^+-channel blockers, it is possible to differentially inhibit these channels. The potassium channels with activation and fast inactivation gating (m^3h, Hodgkin-Huxley kinetics) were blocked by external application of 4 amino-pyridine (4-AP). The potassium channels with standard gating (n^4, Hodgkin-Huxley kinetics) were preferentially inhibited by externally applied tetraethylammonium (TEA). The differential blockage of the two types of potassium conductance changes suggests that they represent two different populations of potassium channels.

It is further shown here that blocking the early transient conductance increase leads to the inhibition of the repetitive electrical activity induced by constant depolarizing current injection in fibers from *Cardisoma guanhumi*.

Key Words giant axon · potassium channel · potassium gating · potassium inactivation

Introduction

In crab nerves from *Carcinus maenas* the time course of the potassium outward current revealed two components: a fast one, with activation-inactivation kinetics, and a delayed one, with kinetics similar to those of the potassium currents recorded in other nerves (Quinta-Ferreira, Rojas & Arispe, 1982). In the preceding paper inward currents recorded in high K^+ (Rb^+ or NH_4^+) artificial seawater were subjected to similar analysis (Quinta-Ferreira, Soria & Rojas, 1985). The time course of the currents, whether carried by K^+, Rb^+ or NH_4^+, could be described in terms of these two components. The excellent fit obtained lent further support to the idea that there are two distinct types of potassium channels in crab nerves.

In this work two potassium channel blockers, namely TEA (Hille, 1967; Armstrong, 1969) and 4-AP (Yeh et al., 1976a,b; Meves & Pichon, 1977), were used to establish further differences between these two components of the potassium conductance.

Materials and Methods

The techniques used in this work have been described in detail in previous publications from this laboratory (Quinta-Ferreira, Arispe & Rojas, 1982a; Quinta-Ferreira et al., 1982b).

Experiments in Norwich were performed on giant axons from the meropodite section of walking legs of *Carcinus maenas* and *Cancer pagirus*. Experiments in Caracas were performed on similar nerve fibers from *Cardisoma guanhumi*.

The nerve chamber, electrode arrangement and feedback amplifier used have been described before (Nonner, 1969; Quinta-Ferreira et al., 1982a). Of the four pools determined by the partitions in the chamber (pools A, B, C and E) only pool A was perfused with artificial seawater (ASW). Table 1 in the preceding paper (Quinta-Ferreira et al., 1985) gives the composition of the solutions used. In experiments with Rb^+ in place of Na^+ in the ASW the concentration of Rb^+ was adjusted to 470 mM. In the text this saline is referred to as Rb-ASW.

Immediately before use 4-AP (Aldrich Chemicals) was added to the ASW from a stock solution.

Results

MEMBRANE CURRENTS IN THE GIANT AXON FROM CARDISOMA GUANHUMI

Figure 1 shows a set of membrane current records made in K-free ASW in the giant axon from *Cardisoma*. The inward currents shown in Fig. 1 are blocked by 300 nM tetrodotoxin (TTX) and the steady-state level of the outward current is not affected. These outward currents are different from those recorded in the giant axon of the squid in that
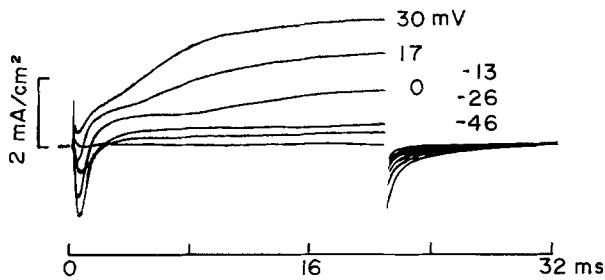
Fig. 1. Voltage-clamp currents in the giant axon of the walking leg from *Cardisoma guanhumi*. Superimposed oscilloscope records of the membrane currents. Experiment 830412A: Fiber diameter = 80 μm. Width of the gap between the Vaseline seals on either side of pool A (here on referred to as A-gap) = 80 μm. Holding potential = −100 mV. Temperature of the K-free ASW in pool A = 20°C. Solution in pools C and E had 450 mM KF, 45 mM NaF, 10 mM Tris Cl at pH = 7.2. Numbers associated with each current trace are the absolute value of the membrane potential during the pulse
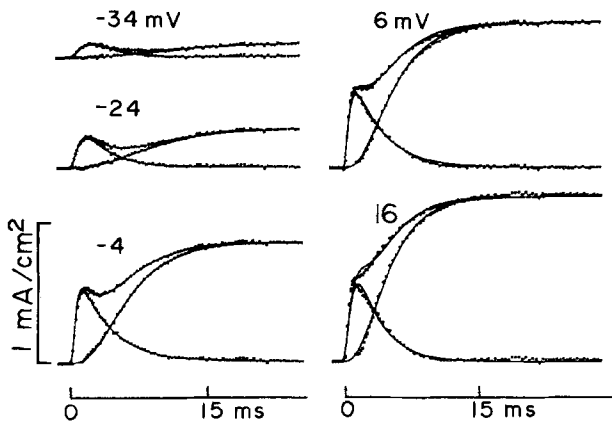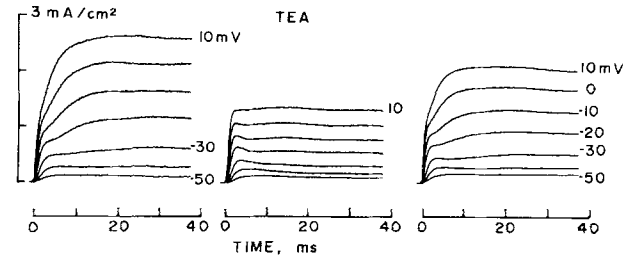

Fig. 3. Effects of external TEA on outward currents in the giant axon from *Cancer pagirus*. Experiment 811120A: Holding potential = −120 mV. Absolute membrane potential during some of the pulses is indicated (10-mV increments between the traces). Fiber diameter = 36 μm. Width of the pool A = 90 μm. Measuring resistance 65,000 Ω. *Left side:* Control run in K-free ASW plus TTX at 17°C with 500 mM KF in pools C and E. *Center:* Effect of external TEA (5 mM). *Right side:* Recovery run after removal of TEA

two kinetic components can be distinguished for positive membrane potentials. After 10 msec the outward current has not reached a steady level. These oscilloscope records, uncorrected for leakage and linear capacity currents, are very similar to the records obtained in axons from *Carcinus* (*see* Fig. 1 in Quinta-Ferreira et al., 1982b). This result, together with previous observations in axons from the crabs *Callinectes sapidus* and *Cancer magister* (Connor, 1975; Connor, Walter & McKown, 1977) and the experiments described in the following sections using axons from two other species, suggests that crustacean nerve fibers in general are equipped with more than one K⁺ conductance system (Connor, 1975; Connor et al., 1977; Quinta-Ferreira et al., 1982b).

Fig. 2. Analysis of the outward potassium currents into an early transient and a delayed sustained component in the giant axon from *Cancer pagirus*. Experiment 811119: Fiber diameter = 40 μm. A-gap = 60 μm. Resistance of the axoplasm of fiber segment from pool E to pool A (here on referred to as measuring resistance) = 60,000 Ω. Solution in pools C and E had 500 mM KF, 10 mM Tris Cl at pH = 7.2 K-free ASW plus 300 nM TTX in pool A. Holding potential (estimated from the position of the Na⁺ conductance inactivation curve assuming that at a resting potential of −70 mV 30% of the channels are inactivated) = −104 mV. Time constants (at 16°C) as follows:

Two Components in the Outward Potassium Current

Using the method of analysis described in Quinta-Ferreira et al. (1982b) one can describe the time course of the outward potassium current recorded from a crab giant axon as the sum of two components. These components are characterized by the following equations:

$$i_{K1} = i_{K1,max}[1 - \exp(-[t - \delta t])/\tau_{n1})]^3 \exp(-t/\tau_{hK}), \tag{1}$$

$$i_{K2} = i_{K2,max}[1 - \exp(-t/\tau_{n2})]^4, \tag{2}$$

and

$$i = i_{K1} + i_{K2} \tag{3}$$

where τ_{n1} and τ_{hK} represent the time constants for the activation and inactivation of i_{K1}, respectively,

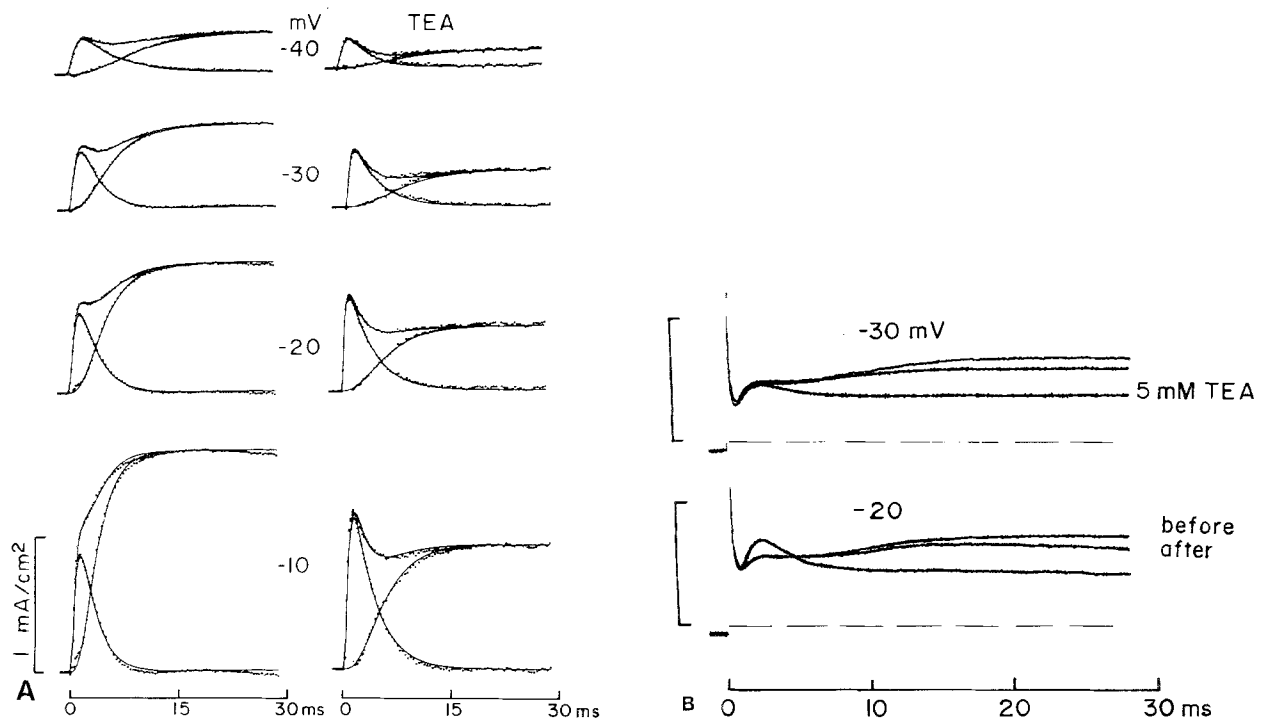| (mV)	τ_{n1} (msec)	τ_{hK} (msec)	τ_{n2} (msec)
−34	1.31	3.02	6.34
−24	0.88	3.01	5.30
− 4	0.42	3.95	3.68
6	0.56	3.59	3.23
16	0.72	2.97	2.87

Fig. 4. Analysis of the effects of external TEA on outward potassium currents in the giant axon from *Cancer pagirus*. *A*. Experiment 811120B: Cut ends of the fiber in 500 mM KF. Holding potential set at −120 mV. Temperature 17°C. *Left side:* Analysis of the outward currents in terms of two components during the control run. Absolute membrane potential during the pulses is indicated next to the corresponding record. *Right side:* Analysis of the outward currents recorded 5 min after the addition of 5 mM TEA to pool A. Resistance of the segment of the fiber from pool E to pool A was 65,000 Ohm measured at the end of this run. *B*. Experiment 811120C: Cut ends of the fiber in 400 mM KF, 50 mM NaF, 20 mM Tris Cl at pH 7.2. Two sets of three superimposed current records are shown. In each set of records the larger current, at the end of the record, was made before the application of TEA. The smallest, during the application of 5 mM TEA, and the record in the middle represents the recovery of the current. Vertical calibrations: 0.5 mA/cm² for the upper set of records and 1 mA/cm² for the lower set of records. Dashed horizontal line represents the estimated leakage current level. The data from this experiment were not included in the Table which presents data obtained with just KF in pools C and E

and τ_{n2} represents the activation time constant of i_{K2}. δt represents a delay in the activation of i_{K1}.

Figure 2 illustrates the analysis of five outward current records obtained from a giant axon of the crab *Cancer pagirus*. The curves shown represent the best fit of Eqs. (1), (2) and (3) to the points. The fit remains satisfactory over the entire range of membrane potentials from −40 up to +50 mV.

Values for the various time constants obtained from the analysis of the current records as presented in Fig. 2 (given in the figure legend) can be compared with those obtained from the analysis of similar data in axons from the crab *Carcinus maenas* (*see* Table 3 in Quinta-Ferreira et al., 1982b). The time constants are similar in both preparations.

EXTERNAL RECEPTOR
FOR TEA IN CRUSTACEAN NERVE FIBERS

In a previous report on the potassium currents in crustacean nerve fibers it was shown that TEA, the specific blocker of the potassium conductance in

nerve (Hille, 1967; Armstrong, 1969; Hille, 1970) and in muscle (Stanfield, 1970), inhibits these currents when applied internally (Quinta-Ferreira et al., 1982b). Internal TEA at a concentration of 5 mM reduced the outward currents measured at 20 msec to 0.3 of their size in the control run. This means that the concentration for 50% blockage (or 50% occupancy of the internal receptors) is less than 5 mM.

Figure 3 illustrates the results from one experiment designed to measure the effects of externally applied TEA on i_{K1} and i_{K2}. The set of membrane current records obtained in the presence of 5 mM TEA are shown in the center. It may be seen that TEA induced a reversible inhibition of i_{K2} to near 50% of its size during the control run (left side). It is also apparent that i_{K1} has been less affected.

In order to determine whether TEA is more effective in blocking i_{K2} than i_{K1}, it is necessary to analyze the outward currents in terms of i_{K1} and i_{K2}. The results from one such experiment are shown in Fig. 4A.

The records made in the presence of 5 mM TEA

Table. Differential blockage of the early transient and delayed potassium conductances by external TEA (5 mM)[a]

Experiment	V_p (mV)	Before		During		After	
		i_{K1}	i_{K2}	i_{K1} (mA/cm²)	i_{K2}	i_{K1}	i_{K2}
811120B	−30	0.43	0.62	0.43 (1.0)	0.29 (0.5)	0.43	0.55 (0.9)
	−20	0.57	0.95	0.72 (1.3)	0.49 (0.5)	0.57	0.85 (0.9)
	−10	0.83	1.62	1.16 (1.4)	0.91 (0.6)	0.85	1.44 (0.9)
811119	−24	0.19	0.2	0.24 (1.2)	0.11 (0.5)	—	—
	−4	0.46	0.69	0.7 (1.3)	0.34 (0.5)	—	—
811112	−30	0.45	1.0	0.45 (1.0)	0.57 (0.6)	0.45	0.72 (0.7)
	−20	0.62	1.2	0.69 (1.1)	0.67 (0.6)	0.62	0.85 (0.7)
811115	−30	0.31	0.65	0.31 (1.0)	0.41 (0.6)	0.30	0.45 (0.7)
	−20	0.41	0.81	0.42 (1.0)	0.51 (0.6)	0.40	0.61 (0.7)

[a] ( ): fractional increase or decrease. Experiments 811120B, 811119 and 811112 fibers from *Cancer pagirus*. Measurements on total membrane current records. All fibers were cut in KF and K⁺-free ASW in pool A.
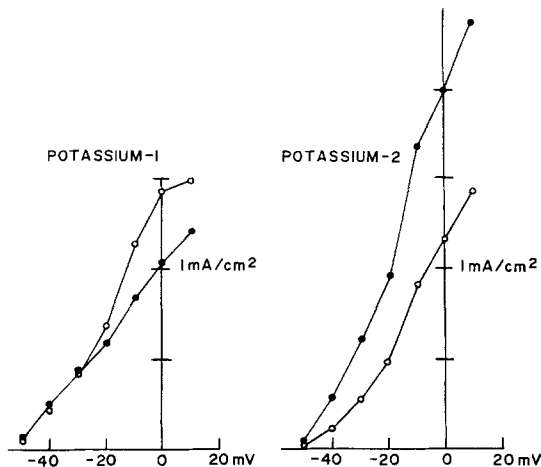

Fig. 5. Effects of TEA on peak i_{K1} and maximum i_{K2}. Data from experiment illustrated in Fig. 4A. Symbols as follows: *Left:* ●: peak value of i_{K1} in K-free ASW plus TTX. ○: peak value of i_{K1} in K-free ASW plus TTX and 5 mM TEA. *Right:* ●: i_{K2} measured at 28 msec in K-free ASW. ○: i_{K2} measured at 28 msec in K-free ASW plus 5 mM TEA

The results from four experiments are summarized in the Table. Two points are clear from these data. The delayed component is inhibited nearly 45% and the early transient component exhibits a marginal increase in the presence of 5 mM external TEA. Furthermore, both effects can be reversed upon removal of the K⁺-channel blocker.

A quantitative analysis of the data from the experiment illustrated in Fig. 4 is presented in Figs. 5 and 6.

The effects of TEA on i_{K1} and i_{K2} are compared in Fig. 5. The time constants for activation and inactivation of i_{K1} and for activation of i_{K2} from the same experiment are presented in Fig. 6.

The data in Fig. 5 clearly show that the external application of TEA selectively inhibits i_{K2}.

The time constants were not affected by the external application of TEA as shown in Fig. 6 suggesting that TEA does not affect the kinetic properties of the gating system. Thus, the TEA selective inhibition of i_{K2} must be caused by a reduction in the number of channels available to conduct current.

shown on the right side of the figure may be compared with the control records shown on the left side. It is immediately apparent that i_{K2} has been reduced to nearly 50% of its size in the absence of TEA. On the other hand, at −20 and −30 mV i_{K1} exhibits a small increase. Figure 4B shows that these effects of TEA are reversible.

Shown in Fig. 4B are two sets of total current records at −30 (upper part) and at −20 mV. It may be seen that while at −30 mV the early transient components is not affected, at −20 mV there is a clear increase in the presence of 5 mM TEA. Notice that i_{K2} was inhibited by externally applied TEA.

TEA Inhibits Inward Rubidium Currents

In the preceding paper (Quinta-Ferreira et al., 1985) we showed that Rb⁺ is a substitute for K⁺ in the potassium systems of these nerve fibers. The experiment presented in Fig. 4 shows a differential inhibition of the outward currents induced by external TEA. In an attempt to induce similar differential blockage of the inward currents we carried out experiments in which Cs⁺ was used in place of K⁺ inside the axon and Rb⁺ in place of Na⁺ in the ASW.
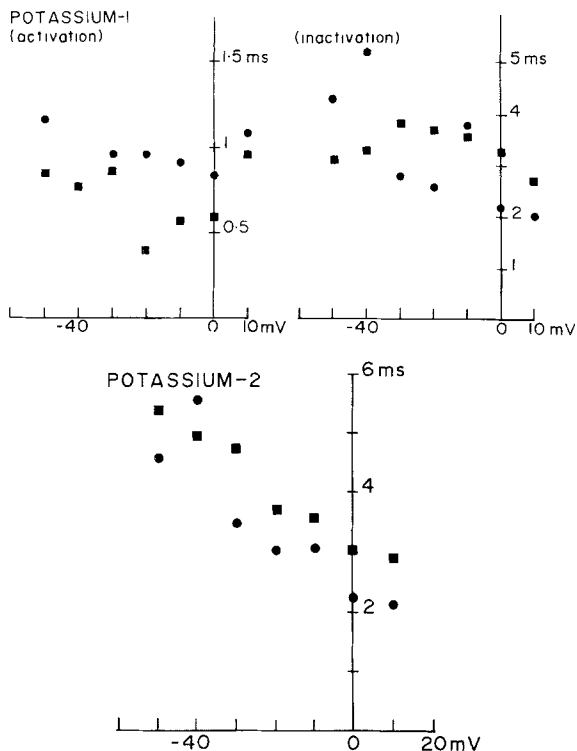
Fig. 6. Time constants for i_{K1} and i_{K2} with and without external TEA. Data from the analysis of the experiment in Fig. 4. *Upper left side:* time constant for the activation of i_{K1}. *Upper right side:* time constant for the inactivation of i_{K1}. *Lower part:* time constant for the activation of i_{K2}. ●: Control in K-free ASW. ■: Run in K-free ASW plus 5 mM TEA
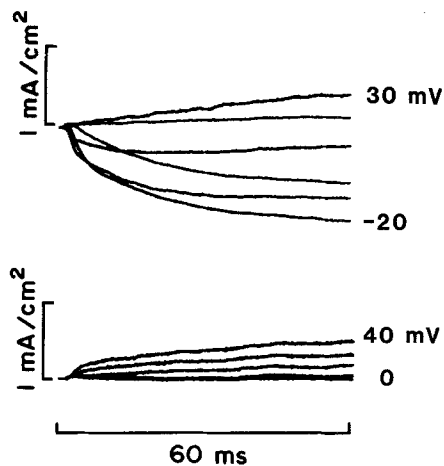

Fig. 7. Effects of external application of TEA on inward Rb⁺ currents in the giant axon from *Carcinus maenas*. Experiment 810419: Holding potential = −80 mV. Absolute membrane potential during the first and last pulses is indicated (10-mV increments between the traces). *Upper part:* Control records made in Rb⁺-ASW plus TTX with 500 mM CsF in pools C and E. *Lower part:* Records made 5 min after the addition of 60 mM TEA to the solution in pool A

Shown in Fig. 7 are two sets of superimposed inward current records made in Rb-ASW with Cs⁺ inside the fiber. The inward currents seen in Rb-ASW plus TTX are effectively blocked by the external application of 60 mM TEA.

It is also apparent in the lower part of Fig. 7 that the remaining outward currents are not affected by 60 mM externally applied TEA. One possible interpretation for this result is that these currents are not specific K⁺-channel currents but leakage currents. If this is the case, then the outward current record at 30 mV shown in the upper part of this figure does not represent Cs⁺ current flowing through the delayed K⁺ conductance system.

The results obtained on reducing the concentration of TEA to 10 mM are shown in Fig. 8. It may be seen that although both components of the inward current are inhibited by TEA, i_{Rb2} is depressed to a greater extent. As for the experiment illustrated in Fig. 7 the outward current is not affected.

A quantitative analysis of the current records in Fig. 8 (in terms of the two components of the inward current (*see* Quinta-Ferreira et al., 1982b) revealed that although both i_{Rb1} and i_{Rb2} are inhibited
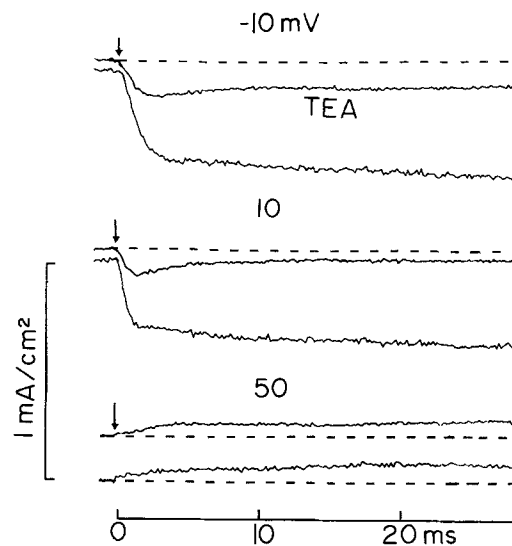

Fig. 8. Effects of externally applied TEA on inward Rb⁺ currents in the giant axon from *Carcinus maenas*. Experiment 810729: Cut ends of the fiber in 500 mM CsF. Holding potential set at −120 mV. Three pairs of records are shown: the upper record in each pair has been displaced in the upward direction and was made after the addition of 10 mM TEA to the Rb-ASW. The lower record in each pair was made in the absence of TEA. Numbers next to each pair represent the absolute membrane potential in mV. The arrows indicate the start of the pulse and the dashed horizontal lines represent the continuation of the base line. Fiber diameter 35 μm; Width of pool A equal to 120 μm; Measuring resistance equal to 220,000 ohm. Temperature 17°C. 300 nM TTX in the Rb-ASW for the two runs
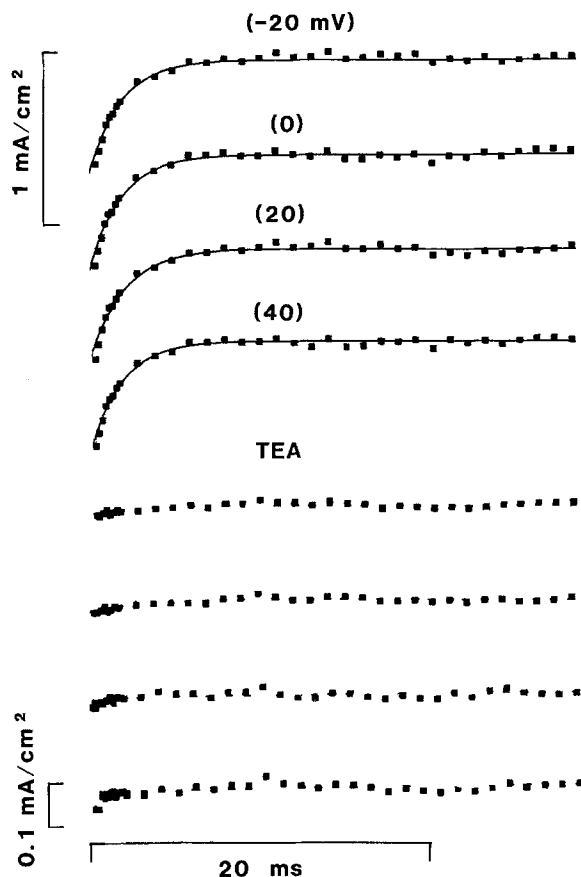
Fig. 9. Analysis of the effects of external TEA on inward current tails. Same experiment as for Fig. 8. Pulse duration about 30 msec. *Top:* Inward current tails recorded in Rb$^+$-ASW plus TTX. Absolute membrane potential during the pulse preceding the tail is given in parentheses next to the tail current. *Bottom:* Tail currents in the presence of 10 mM TEA. Curves represent a single exponential function with time constants in the range from 1.63 to 2.02 msec
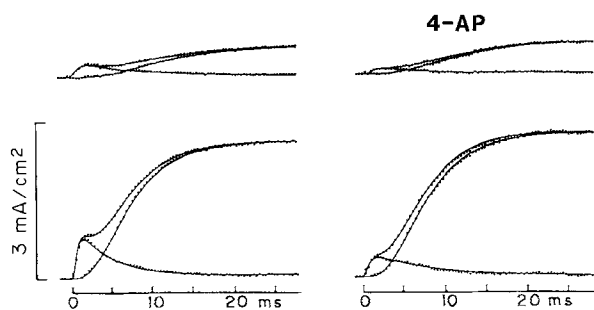

Fig. 10. Analysis of the effects of 4-AP on i_{K1} and i_{K2} in the giant axon from *Cancer pagirus*. Experiment 81114: Holding potential = -114 mV. Width of the pool A = 70 μm. Measuring resistance = 58,000 Ω. Ends of the fiber cut in 500 mM KF. Pool A perfused with K-free ASW plus TTX at 17°C. *Left side:* Analysis of two current records during the control run. Curves represent the least-squares fit of Eqs. (1), (2) and (3) to the points. Membrane potential: -34 (upper) and -14 mV (lower). *Right side:* Analysis of two records of the outward currents recorded in K-free ASW plus 1 mM 4-AP. Membrane potential during the pulses as for the left side
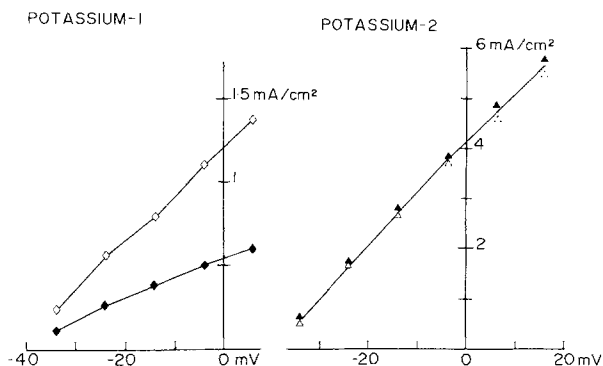

Fig. 11. Effects of externally applied 4-AP on peak value of i_{K1} and maximum value of i_{K2}. Data from the experiment illustrated in Fig. 10. *Left side:* peak values for the transient component. *Right side:* maximum values for the delayed component. ◇: Control run in K-free ASW plus TTX. ◆: Run in K-free ASW plus TTX and 1 mM 4-AP

by TEA, the inhibition of i_{Rb2} is larger. For example, for the pulse to -50 mV (not shown) the ratio i_{Rb1}/i_{Rb2} increased from 0.43 measured in Rb-ASW to 1.7 measured in Rb-ASW plus TEA. At -10 mV the ratio increased from 0.79 to 2.2. As in the case of the inhibition of the outward potassium currents presented in Fig. 4, the time constants of the inward Rb$^+$ currents were not affected by TEA.

The large inward current tails seen upon repolarization (which for pulses lasting more than 15 msec could always be fitted with a single exponential function) were effectively suppressed by TEA. This result is shown in Fig. 9. Although in the presence of TEA the initial values of the tail currents were reduced to less than 5% of their control values, the time constants were not affected. This result also suggests that TEA blocks the current flow through the channels without affecting the gating properties.

EFFECTS OF EXTERNAL APPLICATION OF 4-AP ON THE TWO POTASSIUM CURRENTS

Quinta-Ferreira et al. (1982*b*) reported that external application of 20 mM 4-AP effectively reduced outward currents in the giant axon of the crab *Carcinus maenas* (*see* Fig. 4 in Quinta-Ferreira et al., 1982*b*). A closer inspection of these records showed that at this concentration, 4-AP had completely blocked i_{K1} while i_{K2} had only been inhibited to 30% of its value in the absence of 4-AP. This observation, together with the results on TEA presented here, prompted us to examine the effects of different concentrations of 4-AP.

Figure 10 shows the results from one of the experiments with 4-AP. The membrane currents at -34 and -14 mV in the control run (left side) can be
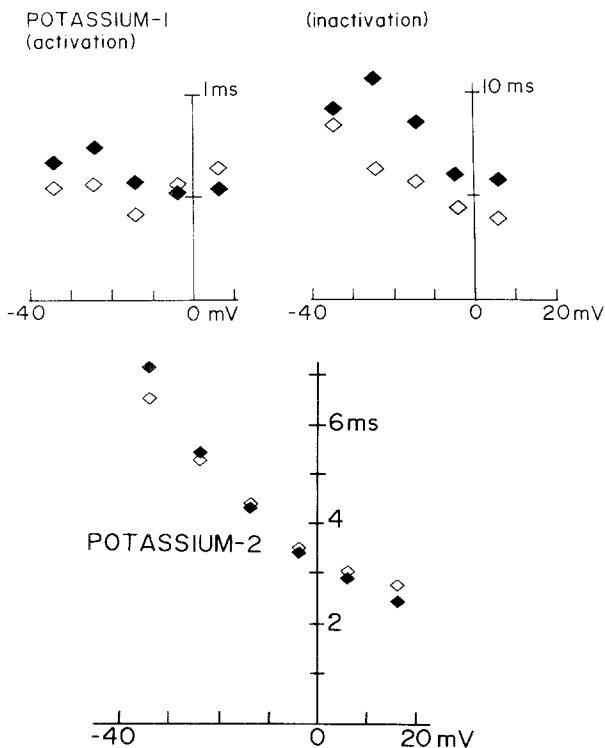
Fig. 12. Time constants for the early transient and delayed sustained component with and without external 4-AP. Data from experiment in Fig. 10. *Upper left side:* τ_{n1}. *Upper right side:* τ_{hK}. *Lower part:* τ_{n2} ◇: Control run in K-free ASW plus TTX. ◆: Run in K-free ASW plus TTX and 1 mM 4-AP
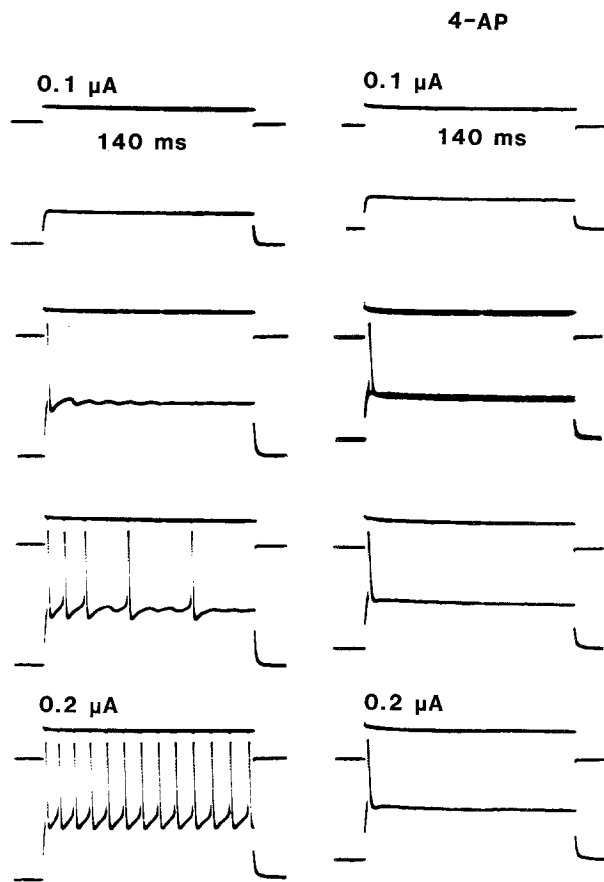

Fig. 13. Effects of 4-AP on the repetitive activity elicited with depolarizing current pulses. Oscilloscope records of the repetitive action potentials in the giant axon from *Cardisoma guanhumi*. Current passing microelectrode tip resistance 10 MΩ; fiber diameter 120 µm. Temperature of the external solution 18°C. In each pair of records, the upper trace represents the current microinjected and the lower the membrane potential. Separation between the microelectrode tips = 480 µm. *Left side:* Membrane responses to depolarizing pulses of current of increasing strength from 0.1 to 0.2 µA with the fiber in K-free ASW. Action potential height 90 mV before the application of 4-AP. *Right side:* Membrane potential responses to pulses of current of increasing intensity from 0.1 to 0.2 µA with the fiber in K-free ASW plus 0.5 mM 4-AP. Pulse duration 140 msec. Resting potential before the application of 4-AP = −68 mV. 20 sec separation between the pulses. Resting potential after 10 min in K-free ASW plus 0.5 mM 4-AP = −58 mV

compared with the membrane currents at the same absolute membrane potentials recorded in the presence of 1 mM 4-AP (right side). The analysis of the currents in terms of i_{K1} and i_{K2} demonstrates the differential effects of 4-AP, i.e., while the peak value of i_{K1} is reduced to nearly 40% of its size in the control run, the maximum i_{K2} value is slightly augmented.

From the analysis of a complete set of membrane current records (like those in Fig. 10) we obtained the i_{K1} and i_{K2} maximum values plotted in Fig. 11.

It may be seen that while the slope of the i_{K1} − V_m curve is reduced from 28.5 to 12.8 mS/cm^2, the slope of the i_{K2} − V_m curve remains constant at 102 mS/cm^2.

The time constants for activation and inactivation of i_{K1} are only moderately affected (Fig. 12). A 55% inhibition of the conductance with small changes in the time constants suggests that the 4-AP molecules block the channels without affecting the gating mechanisms.

Figure 12 (lower part) presents the time constants for the activation of i_{K2} as a function of membrane potential during the pulse. The time constants with or without 4-AP are almost identical; however,

the inactivation time constants in the presence of 4-AP are 30% larger.

Hermann and Gorman (1981) suggested that the blocking action of 4-AP depends on the state of the inactivation gate. Since in these experiments the holding potential was between −20 to −40 mV more negative than the resting potential of −70 mV measured in these fibers (Quinta-Ferreira et al., 1982b), the inactivation gate was in its open conformation.

4-AP INHIBITION OF THE REPETITIVE ACTIVITY ELICITED WITH LONG-LASTING DEPOLARIZING CURRENT PULSES

Figure 13 shows two sets of oscilloscope records of the repetitive action potentials induced by long-lasting depolarizing current pulses in the giant axon from *Cardisoma*. The upper trace in each pair represents the current and the lower trace the membrane response. The records on the left side were made with the fiber in K-free ASW and the records on the right side, with the fiber in K-free ASW plus 0.5 mM 4-AP. Upon removal of the 4-AP from the external saline, the repetitive response observed on the left side of this figure was recovered.

External application of TEA (*data not shown*) did not inhibit the repetitive response.

Discussion

The analysis of the current records in terms of two components with different kinetics presented before (Quinta-Ferreira et al., 1982*b*; Quinta-Ferreira et al., 1985) lent strong support to the hypothesis that crustacean nerve fibers have two different potassium conductance systems (Connor, 1975; Connor et al., 1977). Although definitive proof will require further work, the results presented here strongly suggest that there are at least two different, noninteracting potassium channels in these fibers.

In the present work it was shown that, it is possible to inhibit one of the two components of the outward potassium current using conventional K^+-channel blockers (TEA and 4-AP) provided the concentration is adjusted to maximize the differential effect. The effects of 4-AP on potassium channels have been studied before (Pelhate & Pichon, 1974; Meves & Pichon, 1975; Schauf et al., 1976; Ulbricht & Wagner, 1976; Yeh et al., 1976*a,b*; Dubois, 1981). In all cases 4-AP not only inhibited the potassium system of the cell in which it was tested, but also appeared to affect the gating of the channels. At the concentration used in the present work (in the range from 0.01 to 1.0 mM) the effects of 4-AP presented in Fig. 10 were fully reversible and independent of the pulse frequency used. Therefore, it is safe to conclude that channel gating was not affected by 4-AP in the experiments reported here.

From a graph of the conductance (calculated from records of i_{K1}) as a function of the concentration of 4-AP in the external ASW we calculate a dissociation constant K_d for the 4-AP-receptor complex of 0.47 ± 0.33 mM (mean $\pm$ SD, $n = 4$). Meves and Pichon (1977) reported a potential-dependent K_d increasing from 0.1 to 0.3 mM with the mem-

brane potential during the pulses. For the node of Ranvier K_d is much smaller, i.e., 0.01 mM (Dubois, 1981).

There are several reports in the literature of cell membranes containing more than one potassium conductance. For example, there are three pharmacologically distinct membrane potential gated potassium channels in molluscan neurones (Neher, 1971; Thompson, 1977). In molluscan neurones (Meech, 1974) and in pancreatic B-cells (Atwater, Dawson et al., 1979), in addition to the membrane potential gated potassium channels (Atwater, Ribalet & Rojas, 1979), there are potassium channels which are activated by an increase in the concentration of ionized calcium in the cytosol.

In molluscan neurones there are at least three different potassium systems. Of the voltage-gated systems, 4-AP selectively inhibits the transient K^+ current (50% inhibition with 4-AP in the range from 0.8 to 1.5 mM; Thompson, 1977; Hermann & Gorman, 1981) and TEA blocks the late K^+ current (Connor & Stevens, 1971; Neher & Lux, 1972).

The results presented so far indicate that Na^+ channels in crustacean and in molluscan axons are very similar (Hodgkin & Huxley, 1952*a–d*; Quinta-Ferreira et al., 1982*a*). On the other hand, although both nerve fibers have the slow K^+ channel with n^4 kinetics only the crustacean axons have been provided with a fast K^+-channel which exhibits m^3n kinetics. The presence of this channel in crustacean fibers bears important implications for the transmission of signals along the nerve axon (Connor, 1975). It has been proposed that the transient potassium conductance determines the low-frequency characteristics of the repetitive activity observed in crustacean axons (Connor et al., 1977). The inhibition of the repetitive response induced by the external application of 4-AP as illustrated in Fig. 13 supports this idea.

References

Armstrong, C.M. 1969. Inactivation of the potassium conductance and related phenomena caused by quaternary ammonium ion injection in squid axons. *J. Gen. Physiol.* **50**:491–503

Atwater, I., Dawson, M.C., Ribalet, B., Rojas, E. 1979. Potassium permeability activated by intracellular calcium ion concentration in the pancreatic B-cell. *J. Physiol. (London)* **288**:575–588

Atwater, I., Ribalet, B. Rojas, E. 1979. Mouse pancreatic B-cells: Tetraethylammonium blockage of the potassium permeability increase induced by depolarization. *J. Physiol. (London)* **288:**561–574

Connor, J.A., 1975. Neural repetitive firing: A comparative study of membrane properties of crustacean walking leg axons. *J. Neurophysiol.* **38:**922–932

Connor, J.A., Stevens, C.F. 1971. Inward and delayed outward membrane currents in isolated neural somata under voltage clamp. *J. Physiol. (London)* **213:**1–20

Connor, J.A., Walter, D., McKown, R. 1977. Neural repetitive firing. Modification of the Hodgkin-Huxley axon suggested by experimental results from crustacean axons. *Biophys. J.* **18:**81–102

Dubois, J.M. 1981. Evidence for the existence of three types of potassium channels in the frog Ranvier node membrane. *J. Physiol. (London)* **318:**297–316

Frankenhaeuser, B., Hodgkin, A.L. 1956. The after-effects of impulses in the giant nerve fibers of Loligo. *J. Physiol. (London)* **131:**341–376

Hermann, A., Gorman, A.L.F. 1981. Effects of 4-amino-pyridine on potassium currents in a molluscan neuron. *J. Gen. Physiol.* **78:**63–86

Hille, B. 1967. The selective inhibition of delayed potassium currents in nerve by tetraethylammonium ion. *J. Gen. Physiol.* **50:**1287–1302

Hille, B. 1970. Ionic channels in nerve membranes. *Prog. Biophys. Mol. Biol.* **21:**1–32

Hodgkin, A.L., Huxley, A.F. 1952a. Currents carried by sodium and potassium ions through the membrane of the giant axon of Loligo. *J. Physiol. (London)* **116:**449–472

Hodgkin, A.L., Huxley, A.F. 1952b. The components of membrane conductance in the giant axon of Loligo. *J. Physiol. (London)* **116:**473–496

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

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

Meech, R.W. 1974. The sensitivity of Helix aspersa neurones to injected calcium ions. *J. Physiol. (London)* **237:**259–277

Meves, H., Pichon, Y. 1975. Effects of 4-aminopyridine on the potassium current in internally perfused giant axons of the squid. *J. Physiol. (London)* **251:**60–62P

Meves, H., Pichon, Y. 1977. The effect of internal and external 4-aminopyridine on the potassium currents in intracellularly perfused squid giant axons. *J. Physiol. (London)* **268:**511–532

Neher, E. 1971. Two fast transient current components during voltage clamp on snail neurons. *J. Gen. Physiol.* **58:**36–53

Neher, E., Lux, H.D. 1972. Differential action of TEA on two K-current components of a molluscan neurone. *Pfluegers Arch.* **330:**61–73

Nonner, W. 1969. A new voltage clamp method for Ranvier nodes. *Pfluegers Arch.* **309:**176–192

Pelhate, M., Pichon, Y. 1974. Selective inhibition of potassium currents in the giant axon of the cockroach. *J. Physiol. (London)* **242:**90P

Quinta-Ferreira, M.E., Arispe, M., Rojas, E. 1982a. Sodium currents in the giant axon of the crab *Carcinus maenas*. *J. Membrane Biol.* **66:**159–169

Quinta-Ferreira, M.E., Rojas, E., Arispe, N. 1982b. Potassium currents in the giant axon of the crab *Carcinus maenas*. *J. Membrane Biol.* **66:**171–181

Quinta-Ferreira, M.E., Soria, B., Rojas, E. 1985. Monovalent cation permeabilities of the potassium systems in the crab giant axon. *J. Membrane Biol.* **84:**117–126

Schauf, C.L., Colton, C.A., Colton, J.S., Davis, F.A. 1976. Aminopyridines and sparteine as inhibitors of membrane potassium conductance: Effects on *Myxicola* giant axons and the lobster neuromuscular junction. *J. Pharmacol. Exp. Ther.* **197:**414–424

Standfield, P.R. 1970. The differential effects of tetraethylammonium and zinc ions on the resting conductance of frog skeletal muscle. *J. Physiol. (London)* **209:**231–256

Thompson, S.H. 1977. Three pharmacologically distinct potassium channels in molluscan neurones. *J. Physiol. (London)* **265:**465–488

Ulbricht, W., Wagner, H.H. 1976. Block of potassium channels of the nodal membrane by 4-aminopyridine and its partial removal on depolarization. *Pfluegers Arch.* **367:**77–87

Yeh, J.Z., Oxford, G.S., Wu, C.H., Narahashi, T. 1976a. Dynamics of aminopyridine block of potassium channels of squid axon membranes. *J. Gen. Physiol.* **68:**519–533

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