BRIEF COMMUNICATIONS

INTERACTIONS OF AMINOPYRIDINES WITH POTASSIUM CHANNELS OF SQUID AXON MEMBRANES

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ABSTRACT The effects of aminopyridines on ionic conductances of the squid giant axon membrane were examined using voltage clamp and internal perfusion techniques. 4-Aminopyridine (4-AP) reduced potassium currents, but had no effect upon transient sodium currents. The block of potassium channels by 4-AP was substantially less with (*a*) strong depolarization to positive membrane potentials, (*b*) increasing the duration of a given depolarizing step, and (*c*) increasing the frequency of step depolarizations. Experiments with high external potassium concentrations revealed that the effect of 4-AP was independent of the direction of potassium ion movement. Both 3- and 2-aminopyridine were indistinguishable from 4-AP except in potency. It is concluded that aminopyridines may be used as tools to block the potassium conductance in excitable membranes, but only within certain specific voltage and frequency limits.

Pelhate and Pichon (1974) recently reported that 4-aminopyridine (4-AP) selectively blocked the potassium current in voltage-clamped cockroach giant axons. The block was independent of both membrane potential and direction of current flow. They suggested that 4-AP may become a useful tool for eliminating potassium current in excitable membranes because of its high specificity for potassium channels and its effectiveness upon external application. A thorough understanding of its ionic mechanism of action is of paramount importance prior to acceptance of 4-AP as a useful tool for blocking the potassium channel. We initiated voltage clamp experiments on the squid giant axon to clarify the action of 4-AP and its analogs on potassium channels and to compare our observations on squid axons with those previously reported for cockroach giant axons. Our data confirm some of the previous observations of Pelhate and Pichon (1974), namely selective reduction of potassium currents and induction of spontaneous activity (Pelhate et al., 1972). Additionally and more importantly our data revealed the interaction of amino pyridine analogs with the K^+ channel in exhibit-

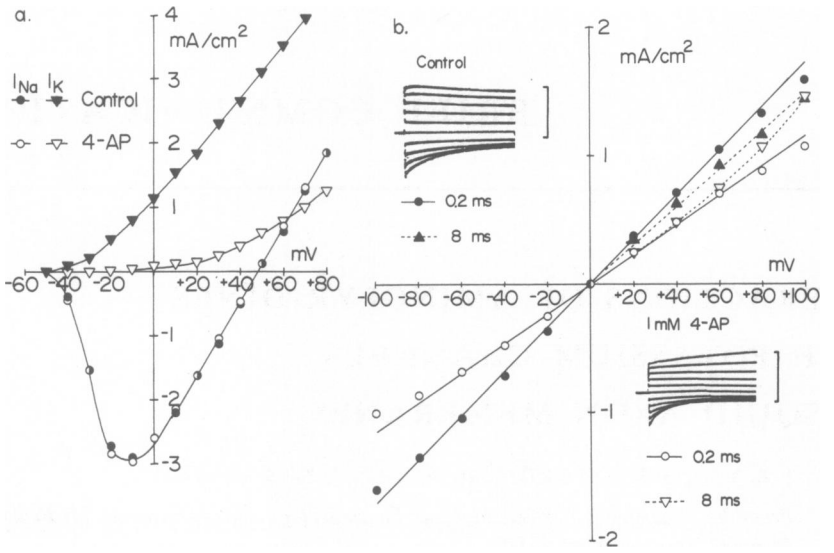

FIGURE 1 Effect of 4-aminopyridine on membrane ionic currents. (*a*) 4-AP (2 mM) was applied internally to an axon bathed in normal potassium seawater (10 mM K^+). The membrane was voltage clamped at −80 mV between step depolarizations which were applied at 30-s intervals to avoid frequency-dependent effects. Potassium currents represent isochronal values measured at 8 ms from the beginning of each pulse. (*b*) 4-AP (1 mM) was applied externally to an intact axon bathed in high potassium seawater (340 Mm K^+). The axon was voltage clamped at the resting potential (0 mV) between depolarizing and hyperpolarizing voltage steps applied every 5 s. Potassium currents represent either quasiinstantaneous values measured 200 μs from the beginning of each pulse (circles) or isochronal values obtained at 8 ms (triangles). The vertical bars for both insets represent 2 mA/cm^2.

ing voltage-, time-, and frequency-dependent characteristics which limit their usefulness as tools.

Experiments were performed on both intact and internally perfused squid giant axons using conventional voltage clamp techniques utilizing double axial electrodes (Wang et al., 1972). The membrane was depolarized by 7 mV on an average (n = 3), and spontaneous firing occurred within minutes after application of 1 mM 4-AP under current clamp conditions. Under voltage clamp conditions 4-AP suppressed potassium current in a dose-dependent manner by either external or internal application. Fig. 1 *a* shows the typical effect of internally applied 4-AP on the membrane ionic current-voltage relation. The potassium current was dramatically suppressed by 2 mM 4-AP, while the transient sodium current was not affected. The degree of suppression of the steady-state potassium current was dependent upon the membrane potential; the block was almost complete at moderate depolarizations, but could be partially overcome by larger depolarizations. This recovery at high depolarizations in the steady-state case is in contrast to the absence of voltage dependency of 4-AP action on cockroach axons as reported previously (Pelhate and Pichon, 1974). The site of action is equally acces-

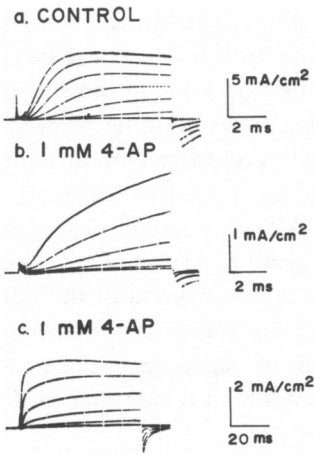

FIGURE 2

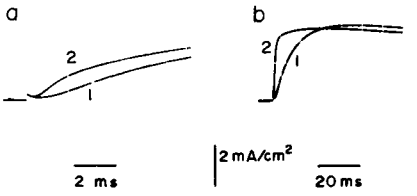

FIGURE 3

FIGURE 2 Time dependency of block of potassium channels by 4-aminopyridine (4-AP) applied externally. The axon was bathed in seawater containing 300 nM tetrodotoxin throughout the experiment to remove transient sodium current and was voltage clamped at −70 mV between depolarizing steps which ranged from −20 to +100 mV. Pulses were applied at 30-s intervals to avoid frequency-dependent effects. Resulting current families are shown before (*a*) and during (*b*, *c*) external application of 1 mM 4-AP. Note different current density and time scales in each set of records.

FIGURE 3 Frequency-dependent component of the 4-aminopyridine (4-AP) block of potassium channels. Axon was bathed in 300 nM tetrodotoxin and 1 mM 4-AP. Two depolarizing pulses to +100 mV were applied with an interval of 1 s. Acceleration of the potassium current is observed during the second pulse. Decrease in steady-state current during the second pulse in record *b* is attributable to accumulation of K^+ in the periaxonal space.

sible to internally or externally applied 4-AP as the effect was identical in terms of onset of action, potency, and specificity for potassium channels.

In order to see the effect of 4-AP on both inward and outward movements of potassium ions, experiments in high external potassium medium were performed. As shown in Fig. 1 *b* potassium ion movements in either the inward or outward direction were suppressed by 4-AP confirming the observation on cockroach axons (Pelhate and Pichon, 1974). While the instantaneous potassium current (circles) was linearly suppressed over the entire voltage range, the decrease in I_K at later times (triangles) exhibited voltage dependency identical to that seen for the steady-state case in normal seawater, suggesting a time-dependent recovery.

Under normal conditions at 10°C, potassium currents reached their steady-state values within a few milliseconds following depolarizing steps over a wide potential range (Fig. 2 *a*). In the presence of 4-AP, however, potassium currents failed to reach final steady-state levels on the same time scale (Fig. 2 *b*) instead requiring several tens of milliseconds (Fig. 2 *c*). Although complicated by accumulation of K^+ in the periaxonal space (Frankenhaeuser and Hodgkin, 1956; Adelman et al., 1973), analysis of

the slow rise of potassium current under 4-AP (assuming negligible accumulation during the short pulse) revealed that simple modifications of the Hodgkin-Huxley n parameter such as voltage shifts, time constant and power function adjustments result in inadequate fits to the kinetics. Because the inhibition appeared to be relieved with a sustained depolarization (Fig. 2 *c*) the slow increase in potassium current is probably due to the progressive removal of 4-AP inhibition during a long depolarization.

The inhibition of the potassium conductance by 4-AP was markedly dependent upon the frequency of stimulation. When twin depolarizing pulses separated by a 1 s interval were applied to the membrane in the presence of 4-AP, the kinetics of the potassium current turn-on associated with the second pulse were much faster than those observed during the first pulse (Fig. 3). Repetitive application of depolarizing pulses progressively accelerated the kinetics of the potassium current until a steady-state pattern was reached.

3-Aminopyridine (3-AP) and 2-aminopyridine (2-AP) exerted similar effects as 4-AP. 3-AP was equipotent with 4-AP whereas 2-AP was the least potent among these aminopyridine analogs. The potencies of these aminopyridine analogs are apparently unrelated to their pK_a values which are estimated to be 9.18, 6.03, and 6.71 for 4-AP, 3-AP, and 2-AP, respectively (Sillén and Martell, 1964).

In conclusion, the effects of aminopyridines on membrane conductances of the squid giant axon meet the criterion of specificity as the potassium channel is selectively blocked. The utility of 4-AP as a tool for electrophysiological studies under voltage clamp conditions may, however, be limited by the complicated time, frequency, and voltage dependencies of action. In addition the spontaneous firing induced by 4-AP in current clamp mode would greatly compromise its utility. The failure to observe voltage-dependent block in cockroach axons (Pelhate and Pichon, 1974) may possibly represent a species difference, however, no critical comparison between preparations can be made as yet for lack of a sufficiently detailed description of 4-AP action in cockroach axons. It still remains potentially useful as a specific blocker of the potassium conductance in high concentrations (500 μM or more) at negative membrane potentials. The effect of aminopyridine analogs on the potassium conductance represents a new interesting interaction of a compound with potassium channels in view of its nonrectifying and time-, frequency-, and voltage-dependent block of the conductance.

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