Short Communication

Use-Dependent Removal of the 4-Aminopyridine-Induced Block of the Transient K^+ Current in Rat Dorsal Root Ganglia

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Abstract. Effects of 4-aminopyridine (4-AP) on the transient K^+ current (I_A) was studied in rat sensory neurons using the whole cell patch-clamp technique. The amplitude of I_A was reduced by 4-AP. The steady-state inactivation curve for I_A was shifted in the positive direction by 4-AP, suggesting that the blocking action of 4-AP may be attenuated by membrane depolarization. When two I_A s were evoked with variable intervals, the peak amplitude of the I_A induced by the second pulse was augmented in the presence of 4-AP. These results indicate that the action of 4-AP can be modulated by concurrent neuronal activities.

Keywords: 4-aminopyridine, dorsal root ganglion, transient K^+ current

Voltage-gated K^+ channels form the largest and most diversified class of ion channels. It is well established that 4-aminopyridine (4-AP) has a pronounced blocking action on the transient type of K^+ current (I_A) (1, 2), and thus this agent has been extensively used as a pharmacological tool in studies of K^+ and other ion channels. However, the mechanism underlying the blocking kinetics of 4-AP has not as yet been fully understood. The voltage and frequency dependence of the 4-AP-induced block of I_A has been described in molluscan neurons (2), dog ventricular muscle (3), and vascular smooth muscle cells (4). We report here use-dependent unblocking of 4-AP-induced block of I_A in mammalian neurons.

The recommendations from the Declaration of Helsinki have been adhered to. Experiments were all carried out in accordance with the Guide for the Care and Use of Laboratory Animals written by the Japanese Pharmacological Society. Dissociated cultures of rat dorsal root ganglion (DRG) neurons were prepared as described previously (5). One- to three-day-old rats were killed by decapitation under ether anesthesia. DRGs were dissected out and incubated at 36°C for 30–40 min in Ca^{2+} - and Mg^{2+} -free saline, containing 0.25% trypsin (Type XI; Sigma, St. Louis, MO, USA). The

ganglia were then mechanically dissociated with a fire-polished Pasteur pipette. The cells were plated on glass cover-slips coated with poly-L-lysine (Sigma) and maintained in a humidified incubator containing 5% CO_2 at 35°C in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum (Gibco Laboratories, Inc., New York, NY, USA), penicillin (40 i.u./ml), and streptomycin (40 ng/ml). After one to two days in culture, cytosine β -D-arabinofuranoside (Sigma) was added to cultures to suppress the growth of non-neuronal cells. Subsequent medium changes were done at a 3- to 4-day interval. Cells between two to ten days in culture were used.

The methods for electrical recording were similar to those previously described (5). Membrane currents were recorded with the whole-cell patch-clamp technique at room temperature (22–23°C). The D.C. resistance of suction pipettes was 1.5–2 M Ω . The pipette solution contained 130 mM K-gluconate, 3 mM Mg-ATP, 2.5 mM $MgCl_2$, 5 mM glucose, 5 mM HEPES, and 5 mM EGTA. The pH of the pipette solution was adjusted to 7.0 with KOH. The external solution contained 120 mM tetramethylammonium-Cl, 5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, and 25 mM glucose. The pH of the external solution was adjusted to 7.4 with NaOH. To minimize the delayed rectifier K^+ current, 5 mM tetraethylammonium-Cl that had no detectable effect on I_A

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was added to the external solution. Since the external solution was Na^+ -deficient, a possible involvement of Na^+ -dependent K^+ current (6) or tetrodotoxin-resistant Na^+ current (7) was eliminated. Tetrodotoxin at $2 \mu\text{M}$ (Sankyo Co., Ltd., Tokyo) was added to block 'outward' tetrodotoxin-sensitive Na^+ current. Ca^{2+} currents were completely blocked by $50 \mu\text{M}$ Cd^{2+} (8). Thus, Ca^{2+} -dependent components of K^+ currents (9) could be eliminated. The liquid junction potential between internal and external solutions (about 11 mV) was compensated by adjusting the zero-current potential to the liquid junction potential. Only cells showing an adequate voltage and space clamp (10) were used. Exponen-

tial fits were determined by computer using a non-linear sum of the least squares fitting routine. Data are expressed as the mean $\pm$ S.E.M., and n represents the number of cells examined.

A holding potential (V_h) of -40 mV completely inactivated I_A , whereas V_h negative to -100 mV fully removed the inactivation of I_A (see the steady-state inactivation curve in Fig. 1C). Using these properties, I_A could be isolated from the total outward current (Fig. 1A). I_A had a fast activating phase and a slow decay phase that was best fitted by a single exponential function (time constant, τ , measured with test pulse (V_t) to -10 mV: 178 ± 15 ms, $n = 5$). The amplitude of I_A was

Fig. 1. Effect of 4-AP on the transient K^+ current (I_A) in rat dorsal root ganglia. A: Isolation of the I_A . A-1: pulse protocol. The test pulse (V_t) was applied either directly from a holding potential (V_h) of -100 mV or following a prepulse (V_{pre}) to -40 mV for 2 s. A-2: a pair of currents evoked with ($I_{pre(+)}$) or without V_{pre} ($I_{pre(-)}$) in the control solution. Upward deflections represent outward currents. In A-3, I_A ($I_{pre(-)} - I_{pre(+)}$) evoked by V_t s from -50 mV to $+10$ mV in 10-mV steps are shown superimposed. B: Current-voltage relationships for I_A in the control solution (open circles) and in the presence of $50 \mu\text{M}$ 4-AP. The peak amplitude of I_A was plotted against V_t . Vertical bars indicate S.E.M. ($n = 5$). C: Steady-state inactivation curves for I_A measured in the control solution (open circles) and in the presence of $50 \mu\text{M}$ 4-AP (filled circles). Two identical step depolarizations to -30 mV for 0.5 s were applied 20 s before (V_c) and immediately subsequent (V_t) to the prepulse (V_{pre}) from V_h of -100 mV. I_t / I_c was plotted as a function of V_{pre} . The plots were compiled by averaging values from 5 measurements. Vertical bars indicate S.E.M. The smooth curves were drawn according to the equation: $I_t / I_c = 1 / (1 + \exp[(V_{pre} - V_{1/2}) / \kappa])$, where $V_{1/2}$ is V_{pre} giving $I_t / I_c = 0.5$, and κ is the slope factor.

reduced to $35.7 \pm 4.1\%$ ($n = 6$) of the control by 4-AP in a concentration of $50 \mu\text{M}$ (IC_{50} , $110 \mu\text{M}$). The peak amplitude of I_A in the presence or absence of 4-AP was plotted against V_t (Fig. 1B). The steady-state inactivation curve for I_A in the control solution had $V_{1/2}$ of -72.7 mV (Fig. 1C, open circles). The curve in the presence of 4-AP was shifted by 11.3 mV in the positive direction (filled circles), although the slope factor was

not affected. Thus, it is suggested that the block by 4-AP may be partially relieved by a membrane depolarization.

Figure 2A shows the recovery process of I_A from inactivation examined by a conventional double pulse protocol. Two identical test pulses (V_c and V_t) were applied with variable intervals (ΔT). In the control solution, the peak amplitude of I_A fully recovered at ΔT of 5 s (open circles). When the medium contained 4-AP, I_A

Fig. 2. Use-dependent unblocking of the 4-AP-induced block of I_A . **A:** Effects of 4-AP on the recovery of I_A from inactivation. Two identical test pulses (V_c and V_t) to -30 mV for 0.5 s were given with variable interval (ΔT) from V_h of -100 mV . The amplitude of I_t normalized to that of I_c was plotted against ΔT . Vertical bars indicate S.E.M. ($n = 5$). **B:** Modulation of the decay phase of I_A by preceding depolarization. A test pulse to -30 mV for 2 s was applied directly (I_c) or 1 s after the prepulse to -30 mV for 0.5 s (I_t). **C:** Effects of 4-AP on the frequency modulation of I_A . From V_h of -100 mV , 9 consecutive test pulses to -30 mV for 0.5 s were applied with variable ΔT , and an additional test pulse (I_{10}) was given after 5 s pause at V_h . **D:** The peak current amplitude for I_{2-10} was normalized to that of I_1 and plotted against the pulse number. Vertical bars indicate S.E.M. ($n = 5$). **E:** A kinetic diagram describing our experimental results (for an explanation, see the text).

evoked by V_t was unexpectedly increased (filled circles). This use-dependent increase of the amplitude of I_A in the presence of 4-AP reached a maximum at ΔT of 1500 ms and declined slowly with a time constant of 7.2 ± 1.7 s ($n = 5$). Thus, the increase of the current was more prolonged than the use-dependent inactivation of I_A in the control solution. The increase was no longer observed at ΔT of 20 s. These results were reproducible in all of the 5 cells examined.

We found in the above experiment that the time course of decay of I_t was accelerated by 4-AP. This point was studied in detail using a longer depolarizing pulse and fixed ΔT (Fig. 2B). The decay phases of I_c and I_t in the control solution could be fitted by a single exponential function (164.2 ± 11.7 ms, $n = 5$ and 162.3 ± 10.7 ms, $n = 5$, respectively). Similarly, the decay phase of I_c in the presence of 4-AP had a single exponential time course (168.2 ± 13.1 ms, $n = 5$). However, I_t in the presence of 4-AP had the decay phase that was described by the sum of two exponentials (time constants: τ_{fast} and τ_{slow} ; 17.2 ± 2.2 ms, $n = 5$ and 160.4 ± 14.6 ms, $n = 5$, respectively). Although τ_{fast} was nearly 10 times smaller than τ_{slow} , the value of τ_{slow} was similar to the time constants in the control solution. Such a modulation of the decay phase by 4-AP was evident also in I_{AS} evoked by repetitive test pulses (see Fig. 2C, lower traces).

Figure 2C illustrates I_{AS} evoked by repetitive test pulses with inter-pulse interval (ΔT) of 640 ms. In the control solution, the peak current amplitude for each pulse in the train was progressively decreased during 9 repetitive test pulses, probably due to an accumulation of inactivated channels (see the time course of recovery from inactivation in the control solution in Fig. 2A). On the contrary, in the presence of 4-AP, each I_A was rather increased, probably due to an accumulation of the fraction of unblocked channels (see Fig. 2A). Results from different ΔT s are summarized in Fig. 2D. It appears that in the presence of 4-AP, there was an interplay between the inactivation process and the increased channel availability due to dissociation of the 4-AP molecules from the blocked channels. I_A evoked 5 s after the termination of 9 repetitive test pulses (I_{10}) was larger than I_1 in the presence of 4-AP, whereas I_{10} in the control solution was identical to I_1 . This may be explained by the finding that at the time of the 10th test pulse, the repriming from inactivation is complete (see open circles in Fig. 2A), while the use-dependent increase of the current is still persistent (see filled circles in Fig. 2A).

Our results clearly demonstrate that a use-dependent facilitation of I_A can be induced by a preceding membrane depolarization in the presence of 4-AP in neurons of the rat DRG. Namely, the peak amplitude of I_A in the

presence of 4-AP was augmented by a preceding membrane depolarization or during repetitive test pulses. Since the use-dependent facilitation declines very slowly, even a single activation of I_A can profoundly affect the 4-AP-induced block of I_A . The frequency of stimulation should not exceed 0.05 Hz to completely avoid the use-dependent effect of membrane depolarization. The positive shift of the steady-state inactivation curve by 4-AP (Fig. 1C) suggest that the use-dependent facilitation of I_A is due to voltage-dependent unblocking of the blocked channels (possibly, voltage-dependent unbinding of 4-AP molecules from the blocked channels).

A striking feature of I_A in the presence of 4-AP was a prominent acceleration of the decay phase accompanying the use-dependent facilitation of I_A (Fig. 2B). Several possible explanations might be considered for the acceleration of the I_A decay. One possibility is that 4-AP binds more strongly to the open (activated) channel state than to the closed state ('open-channel block'). In fact, the open-channel block of I_A has been reported in molluscan neurons (2), in dog ventricular muscle (3), and in neuroblastoma $\times$ glioma hybrid cells (11). However, this possibility may be excluded in the case of rat DRG, since the steady-state inactivation curve was shifted in the depolarizing direction by 4-AP and the preceding activation of channels rather increased the peak amplitude of the current. The possibility that 4-AP preferentially blocks the slower component of 'heterogeneous' I_A (9, 12) may also be excluded, since I_A observed in the present study could be fitted by a single exponential function in the control solution.

Figure 2E represents our kinetic model of I_A in the presence of 4-AP, which describes the use-dependent unblocking action of membrane depolarization and accompanying acceleration of the I_A decay in the presence of 4-AP. At a resting (holding) membrane potential, unbound channels (R) are in equilibrium with 4-AP (D)-bound channels (RD). When the membrane is depolarized, R can open (O) and inactivates (I). At the same time, a fraction of RD can be dissociated back to R^* and D, and R^* can open (O^*) and inactivates (I^*). The two time constants of I_A decay (τ_{fast} and τ_{slow} in Fig. 2B) may reflect faster and slower inactivations of O^* and O, respectively.

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