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4-Aminopyridine (4-AP) Augments Ca^{2+}-dependent Action Potential and Changes Oscillatory Firing Patterns in Rat Cerebellar Purkinje Cells

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Abstract

Intracellular recordings in cerebellar slice preparation showed that applications of 4-AP altered the pattern of oscillatory firing activity in Purkinje cells (PCs), especially yielding pronounced changes in action potential shape. 4-AP increased the amplitude and duration of action potential significantly and decreased the spike frequency. After 4-AP application, the duration of bursting was prolonged and the duration of after-burst hyperpolarization was progressively shortened. In all PCs tested, the rhythmicity of oscillatory firing activity was abolished completely at the steady state. These results suggest that 4-AP-sensitive currents determine the shape and frequency of individual Ca^{2+}-dependent action potentials as well as maintaining oscillatory firing activity in PCs.

Key Words: Purkinje cells, oscillatory firing activity, 4-aminopyridine (4-AP), 4-AP-sensitive current

INTRODUCTION

Purkinje cells (PCs) are capable of generating oscillatory firing activity by direct stimulation or spontaneously, even in the absence of synaptic activity.[1-4,14] Since autorhythmic electrical properties may form the basis for an intrinsic functional context of PCs, the alteration of these properties may be produced by pathological conditions.[1,5,6] Oscillatory activity in PCs is mediated by sequential activation of a set of membrane ionic conductances such as intrinsic Ca^{2+} conductances and Ca^{2+}-dependent conductances.[1,2,4] It is likely that various conductances are simultaneously affected since the extraordinary complexity of the currents underlie the oscillatory activity.

4-aminopyridine (4-AP) is a convulsant in mammals and differs from the most frequently used convulsants such as bicuculline, picrotoxin and penicillin, which block fast synaptic inhibition.[7,8] A general findings with 4-AP in PCs and other neurons is its sensitivity to the fast transient outward potassium current (I_A) at mM concentration.[6-8] I_A is activated by depolarizing steps from holding potentials negative to rest. It is known to participate in frequency encoding of the firing rate in response to graded depolarization, to modulate particularly excitatory synaptic transmission, and to contribute spike repolarizations.[2,9,10] The purpose of this study was to investigate the effects of 4-AP to oscillatory firing patterns of cerebellar PCs in vitro slice preparations.

MATERIALS AND METHODS

Experiments were performed in sagittal cerebellar slices (400 μm thick) of adult Sprague-Dawley rats (80−100 g). Slices were made using a Vibroslice (Electron Microscopy Science, OTS-3000-04) and then submerged in an oxygenated bath containing an artificial cerebrospinal fluid (ACSF). The ACSF consisted of (in mM) NaCl 124, KCl 5, $MgSO_4$ 1.15, KH_2PO_4 1.25, $NaHCO_3$ 26, $CaCl_2$ 2.5, and glucose 10 (pH 7.4). Slices were placed for recording in an interface type chamber that was constantly superfused with ACSF and gassed with 95% O_2/5% CO_2 at room temperature. Intracellular recordings of PCs were performed using micropipettes manufactured with a micropipette puller (Sutter Instrument, P-80). An AxoClamp 2B amplifier (Axon Instrument) was

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used in an active bridge mode during current injection. Current injection and data acquisition were done with pClamp and Axotape program (Axon Instrument). All data were stored on a digital tape recorder (Biologic, DTR 1204), stripe chart recorder (Goulder, TA240), and computer. For all experiments, TTX, which is generally accepted as a synaptic blocker, was applied by microdrop onto the slice at a concentration of 0.3 μM.[1,11] Therefore, action potentials recorded in this study mainly resulted from activities of Ca^{2+} current. 4-AP was dissolved in the superfusion medium.[6,10,12,13] It has been reported that the effect of 4-AP is poorly reversible due to its lipid solubility.[6,7]

Parameters to measure the characteristics of action potential and oscillatory activity were amplitude (mV) and duration (msec) of action potential, frequency of action potential (number/sec), amplitude (mV) and duration (msec) of AHP between spikes, duration of bursting (sec), number of spikes during burst, and amplitude (mV) and duration (sec) of AHP between bursts. The following criteria were used to measure the parameters:[13] the amplitude of action potential was measured as the difference between the point of spike initiation and its peak amplitude; the duration of action potential was measured from the voltage of spike initiation to the point at which the repolarization crossed the same voltage; the duration of AHP between spikes was measured from the end of spike repolarization to the point when the AHP began to decay back to spike initiation; the amplitude of AHP between spikes was measured from the end of spike repolarization to the maximal AHP amplitude; the duration of AHP between bursts was measured from the end of spike repolarization of the last action potential to the point at when the AHP decay back to the initiation of the first spike; the amplitude of AHP between bursts was measured from the end of repolarization of the last action potential to the maximal AHP amplitude. To compare these parameters before and after 4-AP application, the student's t-test was used. Averages are presented with standard error of mean. A p value of less than 0.05 was set for statistical significance in this study.

RESULTS

Effects of 4-AP on the action potential and afterhyperpolarization between spikes

The most common characteristics affected by 4-AP in oscillating PCs was alteration of the action potential shape. In the control period, the average amplitude of action potential was 19.5 ± 10.2 mV (127 observations in the 7 PCs) and the average duration of action potential was 54.7 ± 14.1 ms (94 observations in the 6 cells). Following the addition of 2 mM 4-AP, duration of action potential was prolonged by 22.9%, and the amplitude of action potential was increased by 59.0%, with statistical significance ($p < 0.05$) (Fig. 1A and Table 1).

Application of 4-AP affected the periods of afterhyperpolarization between action potentials in oscillating PCs (Fig. 1B and Table 1). In the control period, the average amplitude of AHP following the action potential was 2.2 ± 1.0 mV (164 observation in 7 PCs) and the average duration of AHP between action potentials was 0.3 ± 0.1 msec (166 observations in 7 PCs). Superfusion of 4-AP produced a 31.8% reduction in the amplitude of AHP and a 33.3% increment in the duration of AHP (Table 1), with statistical significance ($p < 0.05$). 4-AP also decreased

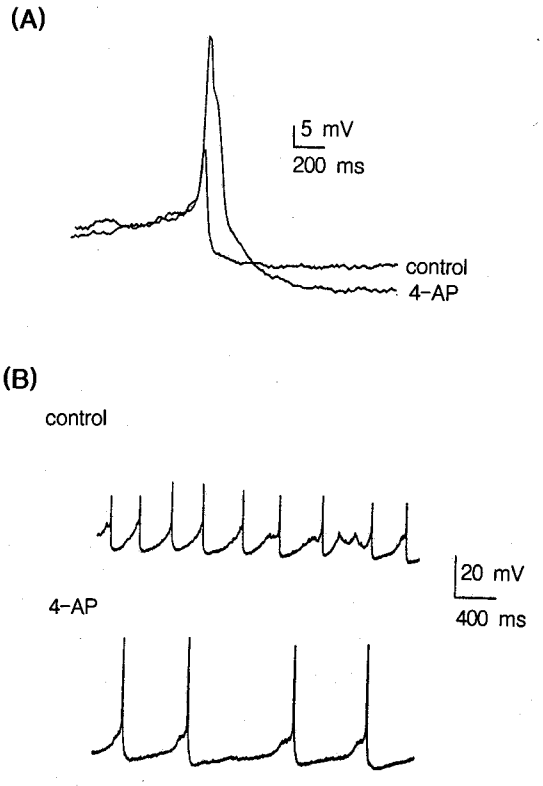

Fig. 1. Effects of 4-AP (2 mM) on the shape and frequency of action potential. (A) Two individual action potentials before and after 4-AP application in same cell are superimposed. 4-AP markedly increased the duration and amplitude of the action potential. (B) 4-AP decreased the firing frequency in a PC. The amplitude of afterspike hyperpolarization was reduced and the duration of afterspike hyperpolarization was prolonged by 4-AP.

Table 1. Effects of 4-AP on Action Potentials

Parameters	Mean±S.E. Before	After	(Change)
Amplitude of action potential (mV)	19.5±10.2	31.0±17.6*	(59.0%)
Duration of action potential (ms)	54.7±14.1	67.2±16.0*	(22.9%)
Frequency of action potential (#/sec)	3.8±0.8	2.4±1.0*	(36.8%)
Amplitude of AHP between spikes (mV)	2.2±1.0	1.5±0.9*	(31.8%)
Duration of AHP between spikes (ms)	0.3±0.1	0.4±0.1*	(33.3%)

Data were analyzed using the student's t-test.
*$p<0.05$.

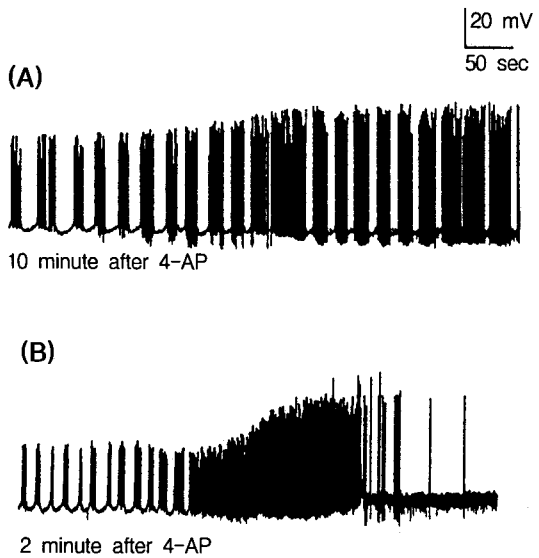

Fig. 2. Effect of 4-AP (2 mM) on oscillatory activity. (A) After 4-AP application, the pattern of oscillatory activity was altered progressively, exhibiting high amplitude oscillation. Consequently, oscillatory activity became irregular, however, spontaneous activities continued (B) 4-AP converted oscillatory firing activity into random firing activity and finally terminated all spontaneous activity. Notice that the membrane potential did not change in panel A and B.

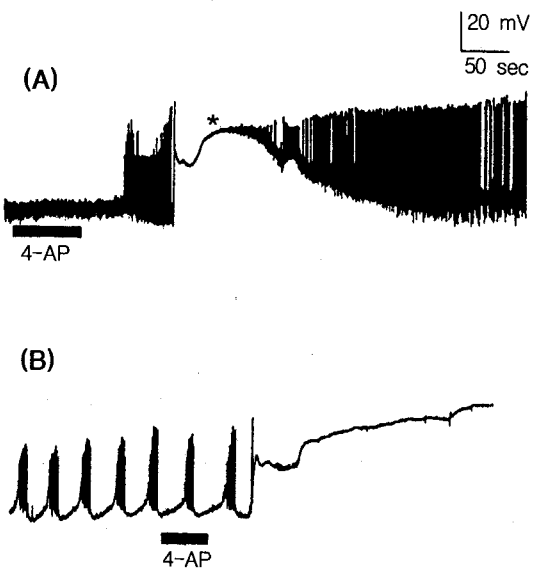

Fig. 3. 4-AP induced a long-lasting, plateau-like depolarization. (A) A non-oscillating PC showed the long-lasting depolarization (*) after 4-AP application. In this PC, the membrane potential was retuned to resting Vm. (B) An oscillating PC displayed a long-lasting depolarization after 4-AP application. The membrane potential did not return to resting Vm, and remain in the depolarizing level as long as recording continued.

the spike frequency by 36.8% ($p<0.05$) (Fig. 1B and Table 1).

Effects of 4-AP on the pattern of oscillatory firing activity

Application of 4-AP to oscillating PCs showed biphasic changes, with complete abolition of oscillatory activity at the steady state (n=10, Fig. 2). Initially, 4-AP changed the oscillatory firing pattern such as action potential shape and duration of burst and afterhyperpolarization between spikes and bursts. Consequently, 4-AP converted regular oscillatory activity to irregular single-spike firing (Fig. 2) or long-lasting, plateau-like depolarization (Fig. 3).

In oscillating PCs, the average duration of bursting was 8.5±5.8 seconds and the average duration of AHP between burst was 15.5±5.8 seconds (Table 2). Following treatment of 4-AP, the duration of bursting was prolonged by 69.4% ($p<0.05$), and the duration of after-burst hyperpolarization was shortened by 21.9% ($p<0.05$). However, the amplitude of AHP between bursts and the number of spikes during bursting were not significantly changed by 4-AP (Table 2). These parameters were collected from 3 bursting sets in 4 oscillating PCs just before oscilla-

Table 2. Effects of 4-AP on Characteristics of Oscillatory Firing Pattern

Parameters	Mean±S.E. Before	After	(Change)
Duration of bursting (sec)	8.5±5.8	14.4±5.3*	(69.4%)
Number of spikes during bursting	29.2±20.5	37.3±14.1	(27.7%)
Duration of AHP between burst (sec)	15.5±5.8	12.1±5.2*	(21.9%)
Amplitude of AHP between burst (mV)	4.7±1.8	3.6±1.3	(23.4%)

Data were analyzed using the student's t-test.
*$p<0.05$.

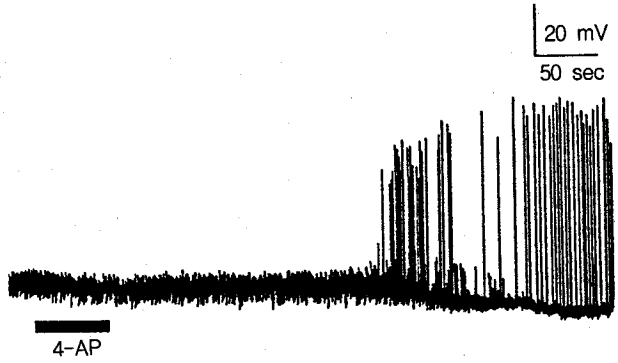

Fig. 4. Initiation of random spiking activity by 4-AP (2 mM) in a quiescent PC.

tory activities were terminated.

Generation of long-lasting, plateau-like depolarization by 4-AP

In the presence of 4-AP, most of the oscillating PCs (9 out of 10) generated a long-lasting, plateau-like depolarization (Fig. 3B) and this effect was also observed in non-oscillating PCs (n=11, Fig. 3A). The long-lasting depolarization exhibited 5 to 60 mV (averaged 27.1±16.8 mV, n=20) positive to the resting membrane potential. During depolarization periods, PCs were able to generate spikes so long as the membrane potential was in the range of spike generation (Fig. 3A). Among PCs which displayed long-lasting depolarization after 4-AP application, 60% of PCs remained at the depolarizing level as long as recording continued (Fig. 3B). However, the membrane potential of the remaining 40% of PCs returned to its resting level even though the firing patterns of spontaneous activity were found to be altered and not recovered (Fig. 3A).

Effects of 4-AP on non-oscillating PCs

We tested the effect of 4-AP in non-oscillating, quiescent PCs (n=12). 4-AP induced random spiking activity in electrically quiescent PCs (8 out of 12, Fig. 4). In theses PCs, the action potentials initiated by 4-AP were also characterized by high-amplitude and long-duration as described above for oscillating PCs. Action potentials initiated by 4-AP did not show any rhythmicity.

DISCUSSION

This study was performed using intracellular recordings from cerebellar slice in vitro. The in-vitro recording method in slice preparation presents technical problems such as mechanical instability and difficulties in modifying the extracellular environment. However, the major advantage of this technique is that the cell body and dentrites of the neuron remain in a healthy state and the intrinsic electrical activities closely resemble those of their counterparts in situ.[3,4,14]

The Ca^{2+}-generating spikes are most prominent in the cerebellar PCs and are basically a dendritic property of PCs.[3,4] In PCs, bursts of action potentials separated by interburst hyperpolarization occurred in a cyclic manner, defined as an oscillatory activity and could be generated spontaneously, which is predominantly Ca^{2+}-dependent activity. It has been known that Ca^{2+}-spikes were caused by the P-type Ca^{2+} channel and repolarized by the BK-type Ca^{2+}-activated K^+ channels in PCs.[4,15] However, it has been suggested that additional potassium currents may contribute generating action potentials in PCs.[15] In the present study, we emphasized 4-AP, an agent well-known for its blocking action of K^+ channels, which are distinct from TEA-sensitive channels.

The most common characteristics affected by 4-AP

in oscillating PCs was alteration of the action potential shape. Following the addition of 4-AP, the duration of action potential was prolonged and the amplitude of action potential increased markedly (Fig. 1 and Table 1). This result suggests that 4-AP-sensitive current is substantially activated at the action potential peak, and normally attenuates the amplitude of action potential. Moreover, the spike-broadening caused by 4-AP commenced at the peak of the action potential (Fig. 1 and Table 1), suggesting that the current blocked by 4-AP has a major role in spike repolarization. Blockade of 4-AP-sensitive current, therefore, enhanced inward current (Ca^{2+} conductances) and resulted in a large increase in duration and amplitude of action potential. It has been shown that TEA-sensitive, sustained outward current, I_K, is also involved in the repolarization of action potential similar to 4-AP-sensitive current.[13] However, I_K is activated later in the repolarization phase and is not involved in the initiation of spike repolarization.[13] The height or width of action potential is a fundamentally important aspect of neuronal activity, such as influencing neurotransmitter release or propagation of impulse through a high-branched axonal or dendritic network.[16]

Application of 4-AP affected the periods of after-hyperpolarization between action potentials in oscillating PCs: a reduction in the amplitude of AHP and an increment in the duration of AHP (Fig. 1 and Table 1). 4-AP also decreased the spike frequency in this study and similar results were reported in cortical neurons,[10] neurons in the gustatory zone of the nucleus tractus solitarius,[17] and in the frog myelinated axon.[18] Decreased spike frequency after 4-AP application could result from slowing the action potential and reducing early accommodation by blockade of repolarizing current. Therefore, it can be suggested that 4-AP-sensitive current in oscillating PCs regulates the firing frequency and pattern of action potential by participating in spike repolarization and hyperpolarizing spike after-potential.[17]

After 4-AP application, the duration of bursting was prolonged and the duration of after-burst hyperpolarization was shortened (Table 2). However, the amplitude of AHP between bursts and the number of spikes during bursting were not significantly changed by 4-AP (Table 2). It has been suggested that a large I_A can be developed during after-burst hyperpolarization since relatively prolonged hyperpolarization can remove inactivation of I_A.[19] The large I_A in this period might retard the rate of depolarization and prolong the interval between bursts.[19] Thus, the depolarization phase of after-burst hyperpolarization might be developed more rapidly after blockade of I_A, and this could explain the decreased duration of after-burst hyperpolarization by 4-AP application as shown in Table 2. Acceleration of the rate of depolarization during after-burst hyperpolarization by 4-AP finally converted rhythmic oscillatory activity to erratic bursting activity (Fig. 2) or prolonged membrane depolarization (Fig. 3B). The mechanism of blocking oscillatory activity after 4-AP application was not related to modulating membrane potential, since this phenomenon was observed without a detectable change in the membrane potential.

In the presence of 4-AP, most oscillating PCs generated a long-lasting, plateau-like depolarization (Fig. 3B) and this effect was also observed in non-oscillating PCs (Fig. 3A). It has been reported that 4-AP produced the plateau-like depolarization for extended periods in rat neostriatal neurons[9] and hippocampal pyramidal cells.[11] It seemed that interfering with repolarizing potassium current by 4-AP could cause such a prolonged depolarization because inward ionic current, with Ca^{2+} conductances, then dominated cell behavior.[7,12] The mechanism of 4-AP as a convulsant is indeed related to the capability of evoking a synchronous depolarizing potential.[11] Hounsgaard and Midtgaard showed that TEA (25 mM) also produced a quiescent plateau phase of depolarization in cerebellar PCs.[20] Therefore, it seems certain that depolarization induced by TEA and 4-AP might result from the blockade of potassium channels which were responsible for membrane repolarization.

We tested the effects of 4-AP in non-oscillating, quiescent PCs. 4-AP induced random spiking activity in electrically quiescent PCs (Fig. 4), and similar phenomena have been observed after applying 4-AP to neurons of sympathetic ganglia[21] and dorsal root axons.[2] Action potentials initiated by 4-AP in quiescent PCs were characterized by high-amplitude and long-duration, which were the same features in oscillating PCs as described above. It has been suggested that much of the I_A was not inactivated at the resting potential in cells which were not spontaneously active.[16] Moreover, the threshold for activation of I_A ranged between -57 and -65 mV in PCs,[6] and the resting membrane potential of PCs in this study ranged from -72 to -52 mV (-62.3 ± 8.5 mV, $n=9$). Therefore, a significant portion of the I_A in quiescent PCs was available for activation at resting membrane potential and was able to reduce the cell's

responsiveness. For this reason, we presumed that 4-AP application to quiescent PCs can easily make cells induce spontaneous firing activity.

In conclusion, 4-AP-sensitive currents play a direct role in determining the shape of action potential and the frequency of spikes, as well as maintaining oscillatory firing activity. The findings of this study suggest that 4-AP, a convulsant, is capable of shaping PC output by modulating firing patterns.

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