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Research report

Sodium-activated potassium conductance participates in the depolarizing afterpotential following a single action potential in rat hippocampal CA1 pyramidal cells

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Abstract

The depolarizing afterpotential (DAP) following an action potential increases the excitability of a neuron. Mechanisms related to the DAP following an antidromic or current-induced spike were studied in CA1 pyramidal cells by whole-cell recordings in hippocampal slices in vitro. In DAP-holding voltage curves, the DAP at 10 ms after the spike peak (DAP10) was extrapolated to reverse at about −50 mV. Increase of extracellular K^+ concentration increased DAP and neuronal bursting. DAP10 reversal potential shifted positively with an increase in $[K^+]_o$ and with the blockade of K^+ conductance using pipettes filled with Cs^+. Similarly, extracellular tetraethylammonium (TEA; 10 mM), 4-aminopyridine (3–10 mM) increased DAP and shifted the DAP10 reversal potential to a depolarizing direction. Decrease of $[Ca^{2+}]_o$ did not alter DAP significantly, suggesting a nonessential role of Ca^{2+} in the DAP. Perfusion of tetrodotoxin (TTX; 0.1–1 μM) and replacement of extracellular Na^+ by choline$^+$ suppressed both spike height and DAP simultaneously. Replacement of extracellular Na^+ by Li^+ increased DAP and spike bursts, and caused a positive shift of the DAP10 reversal potential. It is suggested that Li^+ increased DAP by blocking an Na^+-activated K^+ current. In summary, multiple K^+ conductances are normally active during the DAP following a single action potential.
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Keywords: Depolarizing afterpotential; Sodium-activated potassium current; Synaptic input, Whole-cell recordings

1. Introduction

It has been known for a long time that an axonal spike is initiated by a voltage-dependent Na^+ current and terminated by a delayed rectifier K^+ current [13]. More recent recordings in cortical neurons reveal other currents associated with the spike [16,19]. In hippocampal pyramidal cells, a spike is followed by a depolarizing afterpotential (DAP) [2,10,20] and various afterhyperpolarizing potentials (AHPs) [15,30,32]. The amplitude and duration of the afterpotential, including depolarizing and hyperpolarizing components, are expected to influence neuronal excitability and spike firing patterns for hundreds of milliseconds. Neuronal burst generation, presumably important in neural processing [23], depends on a DAP [17,18].

The DAP in different central neurons may be generated by different mechanisms. In hippocampal CA1 neurons, a non-inactivating, tetrodotoxin (TTX)-sensitive Na^+ current was shown to be responsible for the active DAP recorded in 7.5 mM extracellular $[K^+]_o$ [2]. DAP was increased in low extracellular Ca^{2+} medium, presumably by an increase in the non-inactivating Na^+ current [31].

The participation of K^+ conductances in the DAP has not been systematically studied. An increase in K^+ conductance will hyperpolarize the membrane and decrease the DAP,

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while blockade of K^+ conductance will increase the DAP. Thus, K^+ conductance is important in determining the DAP and excitability. In this study, we studied the participation of various K^+ conductances during the DAP following a single action potential in CA1 pyramidal cells. Both antidromic and current-induced spikes were studied using whole-cell recordings with current clamp.

2. Methods

2.1. Preparation of hippocampal slices

Wistar rats, both male and female, of age ~60 days and body weight ~400 g (Charles River, Quebec, Canada) were deeply anesthetized with halothane and decapitated. The brain was dissected out and placed in ice-cold artificial cerebrospinal fluid (aCSF) for ~5 min, then 450-μm-thick transverse slices were cut from the dorsal hippocampus using a vibratome (Series 1000, Technical Products International, USA). The slices were incubated at room temperature in aCSF oxygenated with 95% O_2–5% CO_2 for at least 1 h before the whole-cell recording.

2.2. Electrophysiological recordings and data analysis

Individual slices were placed in a submerged-type chamber and perfused with oxygenated (95%O_2–5%CO_2) aCSF at room temperature (24 °C) and with a flow rate of 5–8 ml/min. Whole-cell recordings from CA1 neurons were made following procedures described elsewhere [24]. The electrodes had tip DC resistances of 2–5 MW after filling with a pipette solution (below). Signals were sampled at 10 kHz and stored on a microcomputer by a custom-made program, after amplification by an Axoclamp-2A (Axon Instrument, USA) amplifier and low-pass filtered at 3 kHz. Only cells with stable resting potentials <−58 mV and spike overshoots of >15 mV were included in this study. The membrane potential response to step hyperpolarizing currents (HI) of 0.1–0.2 nA was recorded in current-clamp mode. From the latter responses, the input resistance was determined from the steady-state voltage change in response to HI and the time constant was determined by the exponential decay from resting to the hyperpolarizing plateau. Data are expressed as mean±S.E.M. The statistical significance of differences was assessed by ANOVA followed by Newman–Keuls or paired Student's *t*-test.

2.3. Solutions and drugs

The composition of the normal aCSF was (in mM): NaCl 124, KCl 2.4, NaH_2PO_4 1.25, $MgSO_4$ 2, $CaCl_2$ 2, $NaHCO_3$ 26, and glucose 10. The aCSF was maintained at pH 7.4 while bubbled with 95%O_2–5%CO_2. The concentration of K^+, Ca^{2+}/Mg^{2+} and Na^+ in the aCSF was altered in some experiments, but osmolarity was kept constant by adjusting Na^+ accordingly. The typical pipette solution was (in mM): K-gluconate 140, HEPES 10, $MgCl_2$ 4, BAPTA (1,2-bis[2]ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid) 0.1, Na_2-ATP 2, Na_2-GTP 0.2. K-gluconate is known to strongly attenuate the slow AHP in CA1 pyramidal cells [35]. In some experiments, K-gluconate was replaced by Cs-gluconate. The osmolarity of the pipette solution was 285 to 295 mosM and pH was adjusted to 7.3–7.4 with KOH or CsOH.

The following drugs were added to the aCSF depending on the experiment: 4-aminopyridine (4-AP), 6,7-dinitroquinoxaline-2,3-dione (DNQX), kynurenic acid, ouabain, picrotoxin, tetraethylammonium (TEA) and tetrodotoxin (TTX; all from Sigma, USA). Unless otherwise indicated, DAP data were collected with kynurenic acid (1 mM), picrotoxin (0.1 mM) and DNQX (20 μM) added into CSF to block synaptic transmission mediated by glutamate and GABA.

3. Results

3.1. Reversal potential of the DAP

Whole-cell recordings of 83 CA1 neurons yield a resting membrane potential of −63.7±0.4 mV (*N*=83), input resistance 125.7±2.8 MΩ (*N*=83) and time constant 48.8±1.1 ms (*N*=83). Selected staining of some neurons (not shown, but see Liu and Leung [24]) confirmed that they were pyramidal cells. Fig. 1A illustrates the antidromic spike in a CA1 neuron following stimulation of the alveus in a medium with ionotropic glutamatergic and GABA-A receptor antagonists. At rest, the antidromic spike typically reached amplitudes of 100 mV in 0.7–1.1 ms following alvear stimulation (Fig. 1A2). The spike then repolarized quickly but incompletely to a potential positive to rest. This depolarizing afterpotential (DAP) then decayed slowly in ~120 ms (Fig. 1A2). At hyperpolarized voltages (Fig. 1A3), the DAP was large. At depolarized voltages (Fig. 1A1), the DAP was small and occasionally reversed in polarity (became negative instead of positive). The magnitude of the DAP at 10 ms after the spike peak (DAP10) showed a linear voltage relation and extrapolated to a reversal potential of near −50 mV (Fig. 1B, C and D). When the antidromic spike was evoked at a holding potential of −96 mV, which was near the expected K^+ equilibrium potential [24], the magnitude of DAP10 was ~35 mV, and DAP decreased with a positive shift of the holding potential. Typically, a spike could not be activated, and DAP was not measured, at holding potentials of >−45 mV.

A DAP reversal potential of near −50 mV suggests the participation of multiple ionic conductances in its generation. Other than the depolarizing contribution by the non-

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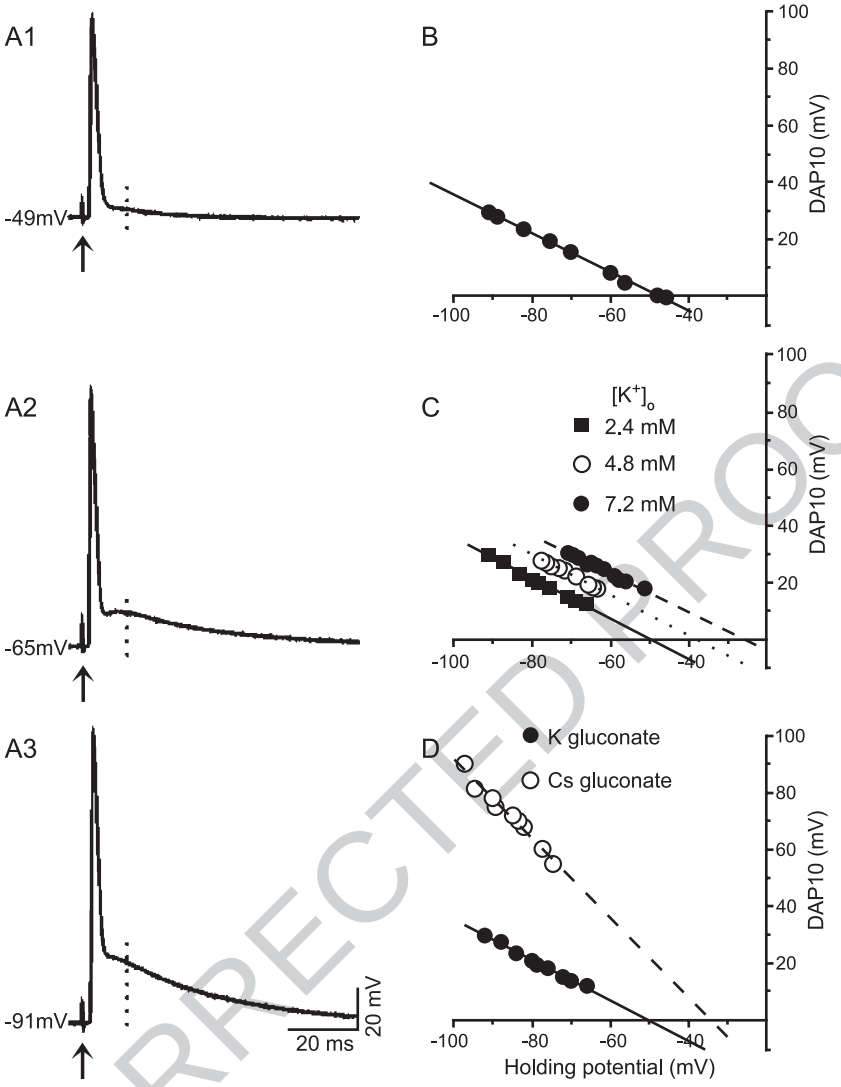

Fig. 1. The amplitude of the depolarizing afterpotential at 10 ms after action potential (DAP10) depends on potassium conductance. (A) DAP10 amplitude increases with holding potential (A1, −49 mV; A2, −65 mV; A3, −91 mV). (B) The relation of DAP10 amplitude and holding potential showing that DAP10 reversed at about −50 mV. (C) DAP10 versus holding voltage plot shifts rightward with increase in $[K^+]_o$ from 2.4 to 4.8 mM and 7.2 mM. (D) DAP10 increased at all holding voltages, and DAP10 reversal shifted rightward with blockade of K^+ conductance by pipettes filled with Cs gluconate (140 mM), as compared to those filled with K-gluconate (140 mM). All recordings were done with kynurenic acid (1 mM), picrotoxin (0.1 mM) and DNQX (20 μM) in the perfusate, and, except for the Cs-gluconate electrode in (D), with a pipette solution containing K-gluconate (140 mM).

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inactivating Na^+ current DAP (Introduction), we suggest that specific K^+ conductances normally provide the hyperpolarization during the DAP.

3.2. Participation of K^+ channels in the afterpotential

The DAP10 was found to increase with a hyperpolarizing holding voltage. The reversal potential of the DAP10 in aCSF with 2.4 mM $[K^+]_o$ was −45±6 mV (n=16). After an increase in $[K^+]_o$ from 2.4 to 4.8 and 7.2 mM, the DAP10 versus voltage curve shifted to the right (Fig. 1C). The DAP10 reversal potential was −24±2.7 mV (n=11) in 7.2 mM $[K^+]_o$.

In perfusate containing ionotropic glutamatergic and GABA-A receptor antagonists, an increase of $[K^+]_o$ from 2.4 to 7.2 mM increased the DAP after a current-induced spike (Fig. 2A1, A2) and the DAP after an antidromic spike (Fig. 2B1, B2). Perfusion of aCSF containing 12 mM $[K^+]_o$ further increased the DAP and resulted in a two-spike burst (Fig. 2A3). A similar DAP with a two-spike burst also appeared during antidromic stimulation (Fig. 2B3). The increase of DAP and the spike bursts were reversed after normal $[K^+]_o$ was restored (Fig. 2A4, B4).

The increase in DAP with increase in extracellular $[K^+]_o$ was found under various conditions. A similar mean percent increase of the DAP10 with high $[K^+]_o$ was found in aCSF containing "antagonists" (glutamate and GABA-A antagonists) and in "high Mg^{2+}/low Ca^{2+}" medium (Fig. 2C and Table 1). However, the percent increase in DAP10

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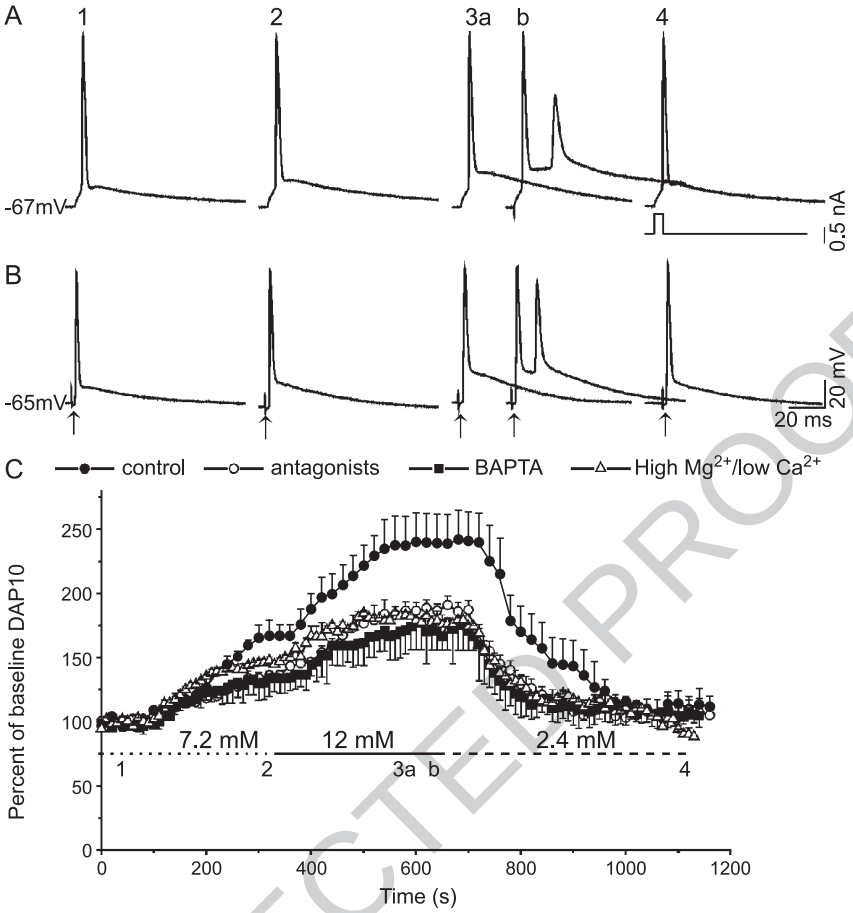

Fig. 2. The amplitude of depolarizing afterpotential at 10 ms after action potential (DAP10) increases with increase in extracellular K^+ concentration $[K^+]_o$. (A, B) Two different CA1 pyramidal neurons perfused with kynurenic acid (1 mM), picrotoxin (0.1 mM) and DNQX (20 μM), and studied during change of $[K^+]_o$. (A) Action potential in a CA1 pyramidal cell induced by a short depolarizing current pulse (0.8 nA, 5 ms, inset under A4) and (B) action potential in another CA1 cell evoked by antidromic electrical stimulation (30 μA, arrows). 1, bathed in 2.4 mM $[K^+]_o$ at the time of switching to higher $[K^+]_o$; 2, near the end of perfusion of 7.2 mM $[K^+]_o$; 3a and b, near the end of perfusion of 12 mM $[K^+]_o$; 4, recovery after the reperfusion of 2.4 mM $[K^+]_o$. 1, 2, 3a and b, 4 correspond, respectively to the positions marked in part C. (C) DAP10 amplitude increased reversibly with an increase in extracellular K^+ concentration. DAP10 amplitude, normalized to 100% during baseline 2.4 mM $[K^+]_o$, was plotted during perfusion of 7.2 (dotted line), 12 mM $[K^+]_o$ (solid line) and return to 2.4 mM $[K^+]_o$ (dashed line). Control group: no antagonists in aCSF, 2 mM Mg^{2+} and 2 mM Ca^{2+}. Antagonists group: kynurenic acid (1 mM), picrotoxin (0.1 mM) and DNQX (20 μM) in the perfusate. High Mg^{2+}/low Ca^{2+}group: Mg^{2+} (6 mM) and Ca^{2+} (0 mM) in perfusate, with kynurenic acid (1 mM), picrotoxin (0.1 mM) and DNQX (20 μM). BAPTA group: 12 mM BAPTA added in pipette solution, perfused with aCSF containing kynurenic acid, picrotoxin and DNQX.

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with high $[K^+]_o$ was significantly larger in normal control aCSF as compared to aCSF with "antagonists" or in "high Mg^{2+}/low Ca^{2+}" medium (Fig. 2C and Table 1). Adding 12 mM BAPTA in the pipette did not prevent the increase of the DAP with raising $[K^+]_o$ ("BAPTA" group in Fig. 2C and Table 1).

The effects of different K^+ channel antagonists on the DAP were studied. Blocking overall K^+ conductance by Cs-gluconate (140 mM) in the pipette increased the DAP and shifted the DAP10 reversal potential in a positive direction, as compared to recordings using K-gluconate filled pipettes (Fig. 1D). In four neurons, 10 mM TEA in the aCSF increased the DAP. This is shown by an increase of DAP10 at all holding voltages <-50 mV, and a positive shift of the DAP10 reversal potential (Fig. 3A). A dose-dependent increase in the DAP was also found after 4AP. 4AP at 3–10 mM also shifted the DAP10 reversal potential to the depolarizing direction, while 0.1 mM 4AP had little effect (Fig. 3B). In contrast, the I_h blocker ZD7288 (50 μM; five cells) or carbachol (100 μM; three cells) was not found to significantly affect the DAP (data not shown).

3.3. DAP increases with replacement of extracellular Na^+ with Li^+

Perfusion of 0–0.2 mM low $[Ca^{2+}]_o$ medium with 4–6 mM Mg^{2+} did not have a significant effect on the DAP (Table 1). The DAP10 reversal potential in low Ca^{2+}/high Mg^{2+} aCSF was -43 ± 2 mV (N=5 cells), not significantly different from the reversal potential in normal aCSF with 2 mM $[Ca^{2+}]_o$.

t1.1 Table 1
Depolarizing afterpotential measured at 10 ms after the peak of action potential (DAP10) increased with extracellular potassium concentration
t1.2 $[K^+]_o$

t1.3 Groups	DAP10 in normal medium (mV)	7.2 mM $[K^+]_o$ (% normal)	12 mM $[K^+]_o$ (% normal)
t1.4 Control	10.0±1.2 (5)	170.8±6.3 (5)#	226.0±13.6 (5)*#
t1.5 Antagonist	10.0±1.9 (5)	137.7±3.2 (5)	181.3±5.8 (5)*
t1.6 High Mg^{2+}/low Ca^{2+}	9.8±2.0 (5)	145.8±1.5 (5)	176.6±2.7 (5)*
t1.7 BAPTA	9.4±2.9 (5)	136.5±6.7 (5)	172.4±15.5 (5)*

The amplitude of DAP10 was measured in normal aCSF containing 2.4 mM $[K^+]_o$, and percent of DAP10 in 7.2 or 12 mM $[K^+]_o$ aCSF, relative to that measured in normal aCSF, is listed. Control group: no antagonists in aCSF. Antagonist group: three antagonists were added to the aCSF—kynurenic acid (1 mM), picrotoxin (0.1 mM) and DNQX (20 μM); they were also added to the aCSF in the high Mg^{2+}/low Ca^{2+} and BAPTA groups. High Mg^{2+}/low Ca^{2+}group: with 6 mM Mg^{2+} and 0 mM Ca^{2+} instead of 2 mM each of Mg^{2+} and Ca^{2+}. The above three groups of neurons were recorded using K^+-gluconate pipette solution (see Methods). BAPTA group: 12 mM BAPTA in the recording pipette solution. Number in parentheses is the number of cells for each measure. The holding potential was of mean −66 and <0.5 mV standard error of the mean for each group
t1.8 (N=5).
t1.9 * P<0.05 while compared to $[K^+]_o$=7.2 mM.
t1.10 # P<0.05 while compared to other groups with the same $[K^+]_o$.

Perfusion of the slices with 50% or 75% of the $[Na^+]_o$ replaced with choline$^+$ (with ionotropic glutamate and GABA-A receptor antagonists) decreased both the antidromic spike height and the DAP at the same time (data not shown), i.e., there was no differential decrease of the DAP without decrease of the spike height. In addition, perfusion of TTX (0.1 to 1 μM in the perfusate) blocked both the spike height and the DAP within minutes, with no differential action on spike height and DAP.

A selective effect on the DAP but not the spike height was achieved by partial replacement of extracellular Na^+ in the perfusate with Li^+. In the presence of ionotropic glutamate and $GABA_A$ receptor blockers, Li^+ replacement of Na^+ increased the amplitude of the DAP without changing the spike height of a CA1 pyramidal cell. The latter was found with the neuron held at a fixed holding potential, for either an antidromic (Fig. 4A) or a current-induced spike (Fig. 4B). Antidromic stimulation may result in a spike burst (Fig. 4A). After partial replacement of extracellular Na^+ with Li^+, the DAP10 reversal potential shifted in the positive direction (Fig. 3C). Li^+ may slightly decrease (Fig. 4A3) or increase the input resistance (Fig. 4B3), but it reliably increased the DAP. Thus, the increase in DAP did not depend on a change in input resistance. Li^+ also depolarized the resting membrane potential by 8.4±1.7 mV (N=16), i.e., when no holding current was applied. The effects of Li^+ on the DAP and resting potential were reversed after wash (Fig. 4A1, B1).

Ouabain, an inhibitor of Na^+/K^+ ATPase, also increased DAP (results not shown). Without hyperpolarizing current injection, neurons depolarized after ouabain, similar to the perfusion of Li^+ aCSF.

4. Discussion

4.1. Na^+-activated and other K^+ conductances participated in the DAP

The original finding in this study is that Na^+-activated K^+ conductance participated in the DAP following a single action potential in CA1 pyramidal cells. This novel result was discovered after we inferred that K^+ conductances are an integral part of the DAP.

After replacing extracellular Na^+ with Li^+, the spike height was normal but the DAP10 was larger and DAP10 reversal potential was shifted to the positive direction. The larger DAP is difficult to explain by an increased participation of Na^+ conductance in the DAP. Lowering $[Na^+]_o$ should decrease the depolarization during the DAP and cause a negative shift of the DAP reversal potential. However, a decrease in K^+ conductance will increase the DAP (near resting) and give a positive shift of the DAP reversal potential. We suggest that replacing extracellular Na^+ with Li^+ blocked an Na^+-activated K^+ (K_{Na}) channel [3,6,7,12,25]. K_{Na} channels are gated by Na^+ on the inside of the membrane, and they may be located near the Na^+ channels [21]. In neocortical pyramidal cells, K_{Na} currents could be activated by a burst of action potentials and by non-inactivating Na^+ channels [7,25]. The onset of the K_{Na} current at <10 ms after a depolarizing voltage step [7] supports its participation during the DAP following a single spike. Extracellular Na^+ replacement and decreasing the Na^+ gradient will also have a secondary effect of decreasing the activity of Na^+/K^+ ATPase and the K^+ gradient across the cell membrane. Indeed, we confirmed that blocking the Na^+/K^+ ATPase by ouabain also increased the DAP, likely by decreasing the K^+ gradient across the membrane and thus shifting the DAP10 reversal potential positively. In any case, the effect of Li^+ replacing extracellular Na^+ on the DAP is explained readily by a decrease in K^+ conductance in the DAP. In addition, the effect of 10 mM TEA and 3 mM 4-AP on the DAP can partially be explained by the effect of these drugs on suppressing K_{Na} currents [3,25].

Sodium-dependent action potentials are inferred to suppress the initiation of Ca^{2+} spikes near the soma of CA1 pyramidal cells [11]. Golding et al. [11] suggested that I_D (blocked by 0.1 mM 4-AP) may control the excitability of the somatic membrane, but it did not participate significantly in the DAP (Results). Instead, the K_{Na} current activated by Na^+ influx is ideal for suppressing Ca^{2+} spikes once an Na^+ spike is initiated.

The reversal potential of the DAP10 at about −50 mV in normal $[K^+]_o$ of 2.4 mM is consistent with the participation of K^+ conductance in the DAP, as further confirmed by perfusion of 4.8–7.2 mM $[K^+]_o$, which shifted the DAP reversal potential to the positive direction. However, the dependence of the reversal potential with $[K^+]_o$ (21 mV for a 3-fold change=44 mV per 10-fold change) was somewhat less than that predicted by the

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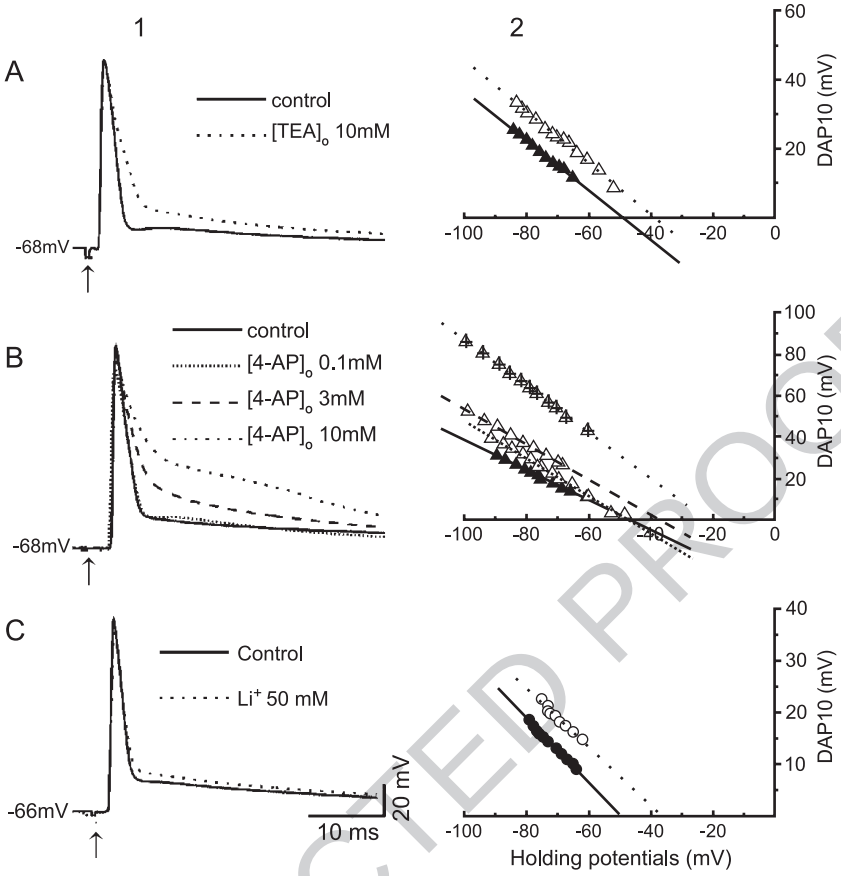

Fig. 3. Potassium channel antagonists 4-AP and TEA and extracellular Li^+ increase the amplitude of DAP10. (A1) TEA (10 mM) increased DAP following antidromic spike evoked by electrical stimulation (arrow). (A2) TEA shifted the DAP10-voltage curve rightward. (B1) 4-AP (0.1, 3, 10 mM), in dose-dependent manner, increased DAP after an antidromic spike evoked by electrical stimulation (arrow). (B2) 4-AP shifted the DAP10-voltage curve rightward. (C1) Li^+ (50 mM) replacing extracellular equimolar Na^+ increased DAP following antidromic spike evoked by electrical stimulation (arrow). (C2) Li^+ replacing Na^+ shifted the DAP10-voltage rightward. All recordings were done in the presence of kynurenic acid (1 mM), picrotoxin (0.1 mM) and DNQX (20 μM).

Nernst equation (59 mV per 10-fold change at 24 °C), possibly because depolarizing ionic (Na^+) conductances also participated in the DAP.

The repolarization of a spike depends on various K^+ conductances [30]. Among them, the slow AHP was greatly reduced in the neurons recorded in this study, since K-gluconate was used in the pipette solution [35]. Adding 12 mM BAPTA to the pipette solution or perfusing with low Ca^{2+} perfusate, which abolished the slow AHP, did not affect the DAP of CA1 neurons significantly (Table 1). In addition, perfusion of carbachol (100 μM) did not significantly affect the DAP, suggesting that I_M or medium AHP [29] did not contribute critically to the DAP. Carbachol (5 μM) [1] or specific I_M blocker linopirdine [33] was reported to suppress an "active" DAP (that formed a hump after the spike) using sharp electrode recording at 33 °C. A difference in recording conditions, including temperature, may underlie the difference in response. It is noted that carbachol may also enhance a plateau potential induced by a long depolarizing current [8].

The K^+ conductances that act to oppose the depolarization during a DAP could be blocked by intracellular Cs^+, extracellular TEA and 4-AP. TEA (10 mM) increased the DAP likely by blocking I_k or other TEA-sensitive K^+ currents (below). A Ca^{2+}-activated K^+ current (I_C) is also expected to be blocked by TEA [27], but I_C (or the fast AHP) may be limited to <10 ms after the peak of the action potential. 4-AP was not effective at a concentration of 0.1 mM, a dose that blocked I_D [28], but higher doses of 4-AP (3 and 10 mM; Fig. 3B) increased DAP and shifted the DAP reversal potential positively, probably by blocking I_A [14]. The latter effect of 4-AP (3–10 mM) on the DAP reversal potential was also found in aCSF with low Ca^{2+} (0.2 mM) and high Mg^{2+} (6 mM), suggesting that dendritic Ca^{2+} spike was not involved (data not shown).

High $[K^+]_o$ increased DAP amplitude more while synaptic inputs were intact than while they were blocked (Fig. 2C). The reason for this is not clear, but it may be partly explained by a reduction of the tonic or evoked GABA-A receptor mediated inhibition because of the decrease of Cl^- gradient across the membrane induced by high $[K^+]_o$ [4,22]. The blockade of GABA-A receptor mediated inhibition during high $[K^+]_o$ may increase the DAP, but only in control aCSF in which GABA-A inhibition was intact. Addition of GABA-A receptor antagonist also increased DAP amplitude in control aCSF (data not shown). In most experiments, the DAP was studied

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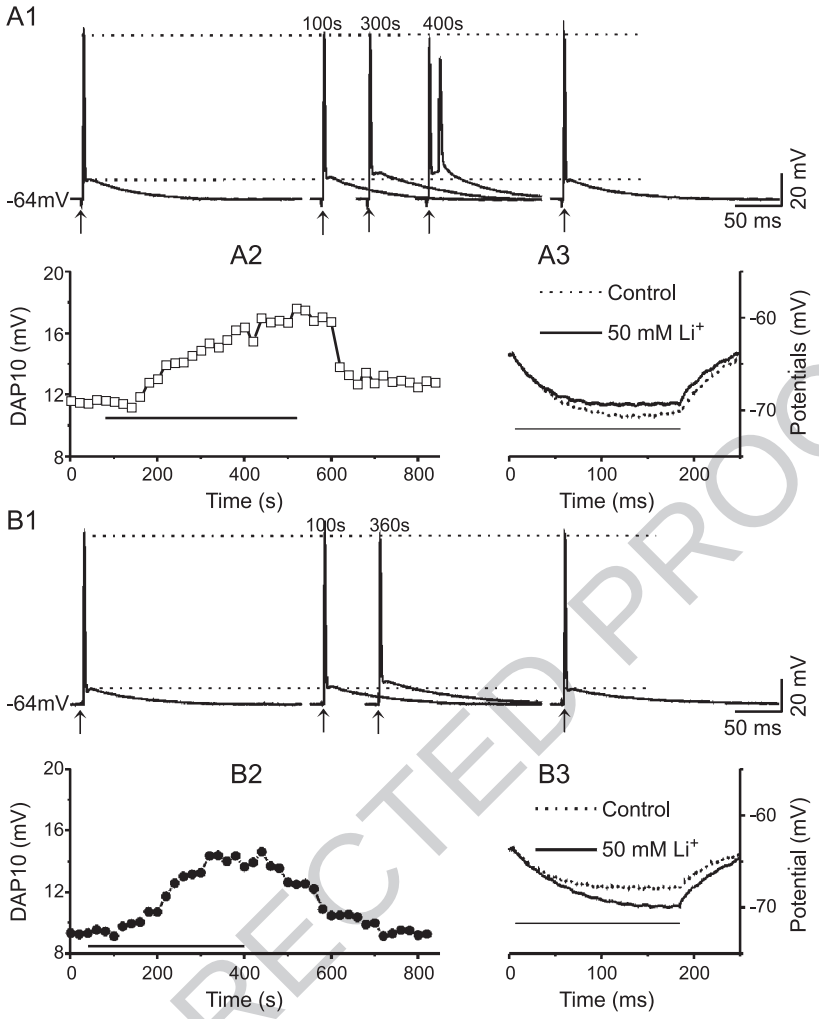

Fig. 4. DAP and bursting increase with partial replacement of extracellular Na^+ with Li^+. (A) The amplitude of DAP in a CA1 pyramidal neuron increased with partial replacement of extracellular Na^+ by Li^+ in the presence of kynurenic acid (1 mM), picrotoxin (0.1 mM) and DNQX (20 μM). (A1) Action potentials evoked by antidromic electrical stimulation (arrows). Left panel, control before Li^+ replacement; middle panel, Li^+ replacement progressively increased DAP and caused cell bursting; right panel, recovery after washout of Li^+. (A2) DAP10 plotted with time, the line indicates time of application of 50 mM Li^+. (A3) Voltage response to a 50 pA hyperpolarizing current step, injection time indicated by the line below. Note the apparent input resistance decreased after 50 mM Li^+. Note DAP increased progressively with the perfusion of 50 mM Li^+ (replacing Na^+) and the effect was reversible (A2 and A1). (B) Same as (A) for another neuron showing similar results, except for an apparent increase in input resistance after 50 mM Li^+ (B3).

in the presence of ionotropic glutamatergic and GABA-A receptor antagonists to exclude the effects of synaptic activity on the DAP.

4.2. DAP contribution by Na^+ and Ca^{2+} currents

Decreasing $[Ca^{2+}]_o$ in the perfusate or chelating intracellular Ca^{2+} by 12 mM BAPTA in the pipette solution did not decrease DAP of CA1 neurons (Table 1). This suggests that Ca^{2+} conductances did not contribute to the DAP, in contrast to hippocampal granule cells [34] or CA3 cells [32]. A non-inactivating Na^+ current [5,9] has been postulated to underlie the depolarizing action of the DAP [2,26,31]. We did not confirm or disprove the presence of a non-inactivating Na^+ current in our neurons, but the presence of an Na^+ conductance, together with K^+ conductances, can explain the reversal potential of the DAP at near −50 mV. Using choline$^+$ to replace extracellular Na^+ reduced spike height and the DAP, and a differential effect on the DAP cannot be distinguished. Similarly, we found that various doses of TTX decreased spike height and DAP at the same time. The latter was in contrast to the result of Ref. [18], which showed that 15 min of perfusion of TTX in an interface chamber blocked the DAP but not the spike height of CA1 neurons. The difference in findings may result from the very rapid medium exchange in our submerged slices (in ~4 min).

In conclusion, we provided several lines of evidence that K^+ conductances normally participated during the DAP that followed a single action potential in hippocampal CA1 neurons. In particular, we suggest the participation of K_{Na} and of K^+ conductances that were sensitive to TEA and 4-AP. These K^+ conductances accompanying or underlying the DAP are important in determining the excitability of neurons.

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