Differential effects of Ca^{2+} channel blockers and Ca^{2+} chelators on currents underlying the late AHP in CA1 hippocampal neurons

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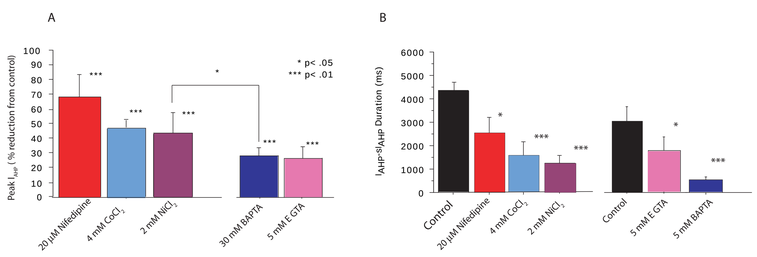

Figure 1. Bath application of Ca^{2+} channel blockers and chelators reduced the AHP tail currents. (A) The peak AHP tail current amplitude is reduced as compared to controls. (B) Effects of the drugs on the duration of I_{AHP} - sI_{AHP}.

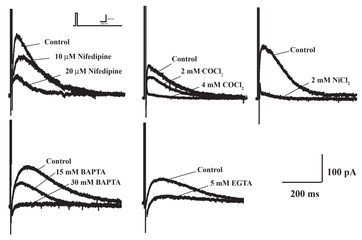

Figure 2. Averaged current traces showing the effects of the drugs on AHP tail currents.

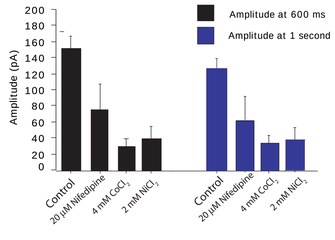

Figure 3. Efffects of Ca^{2+} channel blockers on the amplitudes of the AHP tail currents measured at 600 ms and at 1 sec.

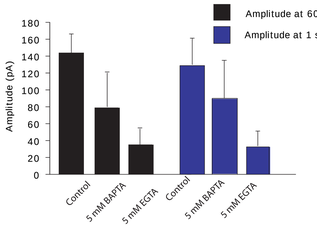

Figure 4. Efffects of Ca^{2+} chelators on the amplitudes of the AHP tail currents measured at 600 ms and at 1 sec.

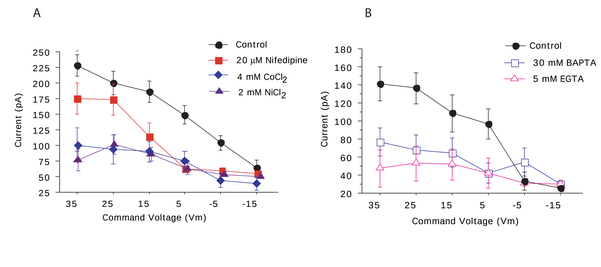

Figure 5. I/V relationships in the presence of Ca^{2+} channel blockers (A) and chelators (B).

Introduction

In hippocampal CA1–CA3 pyramidal neurons, Ca^{2+} entry during action potentials leads to activation of several Ca^{2+}-dependent K+ currents. These currents contribute to the repolarization of the action potential or to one or more phases of the afterhyperpolarization (fast, medium and slow AHP) following single or bursts of action potentials. The medium and late AHPs play a major role in the generation of spike frequency adaptation or accomodation (Alger & Nicoll, 1980; Hotson & Prince, 1980 and Schwartzkroin & Stafstrom, 1980). The late components of the AHP have been suggested to be mediated by four currents: the voltage-dependent I_M (CA1 pyramidal neurons, Storm, 1989), the Ca^{2+}- and voltage-dependent I_C (CA1 pyramidal neurons, Lancaster & Nicoll, 1987 and Storm, 1989), the apamin-sensitive I_{AHP} (bullfrog and rat sympathetic neurons, Pennefather et al., 1985 and Kawai & Watanabe, 1986; neocortical pyramidal neurons, Schwindt et al., 1988a; locus coeruleus neurons, Osmanovic et al., 1990), and the sI_{AHP}, which is modulated by several neurotransmitters and second messenger pathways (Nicoll, 1988 and Sah, 1996). Buffering Ca^{2+} in the cytoplasm by perfusion of BAPTA or EGTA into pyramidal cells has been shown to inhibit the mAHP while low doses of these buffers potentiate the sAHP (Schwindt et al., 1992; Velumian & Carlen 1999; Zhang et al., 1995). External Ca^{2+} channel blockers have been shown to abolish the sAHP suggesting that a rise in Ca^{2+} is required to activate this current. The exact sources and gating mechanisms which regulate the channels underlying the I_{AHP} and sI_{AHP} are unclear. The present study examines the effects of inorganic and organic Ca^{2+} channel blockers and of chelators on the currents underlying the late AHP in CA1 pyramidal neurons.

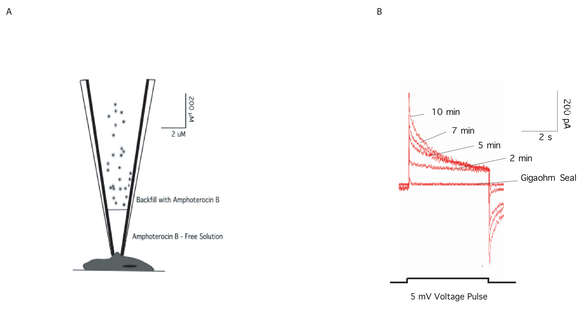

Figure 6. The perforated patch technique. (A).Pippette is front-filled with intracellular solution up to 200 µM of the tip and then back -filled with Amphoterocin B to prevent the tip from clogging. (B). Current traces taken over a 10 min period showing the perforation of Amphoterocin B after gigaohm seal formation.

Methods

Subjects: Fifty male LE rats (2-4 mo) were housed in a controlled laboratory with a 12 h light/dark cycle, with food and water available ad lib. Animal care procedures were performed in compliance with the current Animal Care Committee regulations of the University of Texas at Dallas and guidelines of the USDA.

Drugs: nickel chloride, cobalt chloride, BAPTA, EGTA, nifedipine, TEA, TTX were purchased from Sigma (St. Louis, MO).

Slice preparation: Rats were sacrificed and decapitated under isoflurane anesthesia. Brains were rapidly removed, and hippocampi dissected out using standard procedures (Thompson et al., 1996; Gant & Thompson, 2001). Following removal, brains were immersed in cold (0°C) oxygenated (95% O_2 - 5% CO_2) sucrose artificial cerebrospinal fluild (aCSF) solution which contains 184 mM of sucrose for NaCl (see aCSF composition below, and Aghajanian & Rasmussen, 1989). Coronal slices (300 µm thick) were sectioned using a vibratome and incubated for 1 hr at room temperature (~23° C) in a holding chamber with normal aCSF containing (in mM) 124 $NaCl_2$, 3 KCl, 1.3 $MgSO_4$, 1.24 NaH_2PO_4, 2.4 $CaCl_2$, 26 $NaHCO_3$, 10 D-glucose. Slices were transferred to a submersion chamber for recording and continuously perfused with aCSF at 25° C prior to use.

Patch electrodes and solutions. Patch electrodes were pulled using a Brown-Flaming micropipette puller (Sutter Instruments, Novato, CA) and heat-polished with a Narishige microforge (Model MF-930; Narishi International USA, Inc., East Meadow NY) to a resistance of 3-6 MΩ and filled with a fresh solution consisting of the following (mM): $KMeSO_4$, 126; KCl, 14; Hepes, 10; $MgCl_2$ 3,. For whole-cell, the solution consisted of the following (mM): 40 Tri-ATP, 3 Tris-GTP, 5 Hepes, 140 $KMeSO_4$ The methylsulphate anion was used because it has been shown to preserve sI_{AHP} in a form similar to that observed with sharp electrode recordings (Lancaster & Adams, 1986; Zhang et al. 1995). The solutions were adjusted to 7.25 pH with KOH and to 290 ± 10 mOsm. Amphoterocin-B (final concentration of ~ 150 µg/ml) dissolved in 100 % DMSO was added to the filtered pipette solution prior to recording.

Cell Selection and Data Acquisition. Hippocampal neurons were visualized using a Leica DM LFS microscope (Leica Micro Systems AG, Welzlar, Germany) equipped with long working distance 40x water immersion objective and infrared differential interference contrast optics. Recordings were made from the soma of visually identified CA1 pyramidal neurons with seal resistances >3 GΩ before going into the perforated patch mode. Whole-cell configuration was obtained by rupturing the membrane patch. Open pipette resistances were 2-4 MΩ. The electrical access was indicated by an increase in the current flow (> 500 pA) after 2-5 min or a final access resistance of 10-15 MΩ. Series resistance compensation was set to 30-40 % with a 10 µs lag. Data were acquired at 5 kHz using a National Instruments PCI MIO-16E A/D board (National Instruments, Austin, TX) and filtered at 2 kHz using a low-pass Bessel filter. Membrane currents were recorded and filtered (1 kHz) using an Axopatch 200B (Axon Instruments, Foster City, CA, USA). Series resistance and capacitance were measured and compensated shortly after the perforated patch or whole-cell mode was achieved. Data were acquired online using LabView and NIDAQ (National Instruments, Austin, TX) and off-line analysis was performed using Igor Pro (WaveMetrics Inc., Lake Oswego, OR) and StatView (SAS Institute Inc., Cary, NC). Differences in kinetic properties and drug-related responses were analyzed using ANOVAs, and post hoc Sheffe's were used to test for main effects. Data are reported as mean ± SEM. A neuron was chosen for analysis only if it had series resistance <15 MΩ, membrane resistance > 35 MΩ, and resting potential < - 50mV.

Pharmacological isolation. Baseline recordings of the currents underlying the late AHP were measured in modified aCSF solution containing 500 nM tetrodotoxin (TTX) to block Na^+ currents and 5 mM tetradotoxin (TEA) to block voltage-dependent K^+ currents. All drugs were applied by switching to a superfusion fluid containing the drug using a multi-way tap.

Voltage clamp protocols. The currents underlying the late AHP were evoked by a 100-ms voltage step to 0 mV from a holding potential of - 55 mV. The -55 mV holding potential eliminated the influence of I_H, which activates at more hyperpolarized potentials.

Results

Recordings were obtained from 109 CA1 neurons (35 whole-cell and 74 perforated-patch, respectively). Overall, BAPTA and EGTA produced greater percentage reductions in the peak amplitude of the I_{AHP} in CA1 neurons as compared to Ca^{2+} channel blockers (Fig.1A). These effects were not due to differences in passive membrane properties. Even at saturating concentrations of bath-applied nifedipine, an L-type channel blocker, the reductions in the peak amplitude of the I_{AHP} was less than the other drugs tested. Nifedipine, along with the other Ca^{2+} blockers and chelators, however, significantly reduced integrated area and duration of the I_{AHP} and sI_{AHP} . Durations of I_{AHP} and sI_{AHP} were significantly reduced after bath application of 20 µM nifedipine, 4 mM $CoCl_2$, and 2 mM $NiCl_2$ ($p < 0.03$) (Fig.1B). For the chelators, the reductions in the duration were also significant at both 15 mM and 30 mM of BAPTA ($p < 0.02$;$p<0.001$, respectively). There was a trend toward reduction in the duration of the I_{AHP} and sI_{AHP} following bath application of EGTA . The duration of the I_{AHP} and sI_{AHP} in the presence of TTX and TEA lasted ~ 5 sec after the pulse offset (Fig. 2). However, following bath application of drugs, the duration of the I_{AHP} and sI_{AHP} was significantly reduced to less than 1.5 sec, especially at saturating concentrations of the $CoCl_2$, $NiCl_2$ EGTA, and BAPTA. Clearly, these Ca^{2+} blockers and chelators abolished the sI_{AHP} since its decay time constant is ~ 1.5. A notable difference between the two recording modes was the latency to peak of the I_{AHP}; it was slower in whole-cell than in perforated patch mode. Amplitudes of the tail current measured at 600 ms and 1 s showed similar reductions following drug applications, suggesting that these drugs affect both the I_{AHP} and sI_{AHP} (Fig. 3 and 4). Increasing the voltage command step to more depolarizing potentials produced larger tail currents (Fig. 5). In the presence of inorganic blockers or chelators the reduced Ca^{2+} influx reduced the slope of this curve.

Summary

- All drugs tested reduced I_{AHP} and sI_{AHP}.
- L-type channels play a small role in the generation of I_{AHP} and sI_{AHP}.
- This study contributes to a better understanding of the currents underlying the AHP in a more intact preparation, the perforated patch.