Introduction

In hippocampal CA1–CA3 pyramidal neurons, Ca2+ entry during action potentials leads to activation of several Ca2+-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 accommodation (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 Ia (CA1 pyramidal neurons, Storm, 1989), the Ca2+ - and voltage-dependent l (CA1 pyramidal neurons, Lancaster & Nicoll, 1987 and Storm, 1989), the amphoteric-sensitive i (Buhr & rat sympathetic neurons, Pennefather et al., 1985 and Kawai & Watanabe, 1986, neocortical pyramidal neurons, Schwidt et al., 1988a, locus coeruleus neurons, Osmanovic et al., 1990, and the sl, which is modulated by several neurotransmitters and second messenger pathways (Nicoll, 1988 and Sah, 1998). Buffering Ca2+ in the cytoplasm by perfusion of BAPTA or EGTA into pyramidal cells has been shown to inhibit the mAHP while leaving the NaNAAH and sAHP (Schwindt et al., 1992; Velumian & Carlen, 1999; Turrigiano et al., 2000) and Na+, KNAAH 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 mM. Amphotericin-B (final concentration of ~ 0.5 mM) was added to the filtered pipette solution prior to recording.

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: nelson 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% O2, 5% CO2) sucrose artificial cerebrospinal fluid (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 h at room temperature (~23°C) in a holding chamber with normal aCSF containing (in mM) 124 NaCl, 3 KCl, 1.3 MgSO4, 1.24 NaH PO4, 2.4 CaCl2, 26 NaHCO3, 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, Narishige International USA, Inc. East Meadow NY) to a resistance of 3–6 MΩ and filled with a fresh solution consisting of the following (mM): KMeSO4, 126; KCl, 14; Hepes, 10; MgCl2, 2 (p < 0.03) (Fig.1B). For the chelators, the reductions in the AHP tail 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 amplitudes of the AHP tail currents measured at 0.2, 0.4 and 1.0 sec. Amplitudes were normalized by recording the tail current at 0 mV and subtracting the contribution of the IaHP.

Baseline recordings of the currents underlying the late AHP were measured in modified aCSF solution containing 500 nM tetrodotoxin (TTX) to block Na+ spikes recorded in the absence of TTX and TEA lasted ~5 sec after the pulse offset (Fig. 2). Following bath application of drugs, the duration of the IaHP and IsIAHP was significantly reduced to less than 1.5 sec, especially at saturating concentrations of the CoCl2. The IaHP after recovery periods of different duration in TTX and TEA last ~5 sec after the pulse offset (Fig. 2). Following bath application of drugs, the duration of the IaHP and IsIAHP was significantly reduced to less than 1.5 sec, especially at saturating concentrations of the CoCl2. Differences in kinetic properties and drug-related effects were analyzed using ANOVAs, and post hoc Sheffe’s were used to test for main effects. Data are reported as mean ± SEM. A neurone 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 mM tetrodotoxin (TTX) to block Na+ currents and 5 mM tetrodotoxin (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.

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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 IaHP in CA1 neurons as compared to Ca2+ 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 IaHP was less than the other drugs tested. Nifedipine, along with the other Ca2+ blockers and chelators, however, significantly reduced integrated area and duration of the IaHP and IsIAHP. Durations of IaHP and IsIAHP were significantly reduced after bath application of 20 µM nifedipine, 4 mM CoCl2 and 2 mM NiCl2 (< 0.03) (Fig.1B). For the chelator, the reductions in the IaHP and IsIAHP was significantly reduced to less than 1.5 sec, especially at saturating concentrations of the CoCl2. Nifedipine, and the other Ca2+ blockers and chelators, however, significantly reduced integrated area and duration of the IaHP and IsIAHP. Durations of IaHP and IsIAHP were significantly reduced after bath application of 20 µM nifedipine, 4 mM CoCl2 and 2 mM NiCl2 (< 0.03) (Fig.1B). For the chelator, the reductions in the IaHP and IsIAHP was significantly reduced to less than 1.5 sec, especially at saturating concentrations of the CoCl2. Differences in kinetic properties and drug-related effects were analyzed using ANOVAs, and post hoc Sheffe’s were used to test for main effects. Data are reported as mean ± SEM. A neurone was chosen for analysis only if it had series resistance <15 MΩ, membrane resistance >35 MΩ and resting potential < -50mV.

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Summary

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