Oxidative Downmodulation of the Transient K-Current I_A by Intracellular Arachidonic Acid in Rat Hippocampal Neurons

KATRIN BITTNER AND WOLFGANG MÜLLER
AG Molekulare Zellphysiologie, Institut für Physiologie der Charité, D-10117 Berlin, Germany

Bittner, Katrin and Wolfgang Müller. Oxidative downmodulation of the transient K-current I_A by intracellular arachidonic acid in rat hippocampal neurons. *J. Neurophysiol.* 81: 508–511, 1999. Membrane-permeable arachidonic acid (AA) is liberated in a Ca^{2+}-dependent way inside cells. By using whole cell patch clamp we show that intracellular AA (1 pM) selectively reduces I_A in rat hippocampal neurons, whereas extracellular application requires a 10^6-fold concentration. The nonmetabolized AA analogue ETYA mimics the effect of AA that is blocked by ascorbic acid or intracellular glutathione, suggesting an intracellular oxidative mechanism. We conclude that intracellular AA is extremely potent in reducing I_A by an oxidative mechanism, particularly during oxidative stress.

INTRODUCTION

Arachidonic acid (AA) is part of an ubiquitous second-messenger system that has been implicated in transmembrane actions in synaptic plasticity and brain damage (Okada et al. 1989; Williams and Bliss 1988, 1989). AA is released from the membrane primarily by G-protein– or calcium (Ca^{2+})-mediated activation of phospholipase A_2 (PLA_2) (Axelrod et al. 1988; Burch 1989; Chang et al. 1987; Kim et al. 1989). Stimulation of *N*-methyl-D-aspartate (NMDA) receptors is particularly effective in releasing AA (Dumuis et al. 1988), probably because of highly localized Ca^{2+} signals (Müller and Connor 1991).

AA is rapidly metabolized to a variety of bioactive messengers, e.g., prostaglandins, leukotrienes, prostacyclins, and thromboxanes. AA itself as well as metabolites affect a wide variety of ion channels, including sodium-, potassium-, calcium-, chloride-, and ligand-gated channels either directly or by mechanisms including oxidation and protein kinase C (PKC) (Anderson and Welsh 1990; Keyser and Alger 1990; Linden and Routtenberg 1989; Miller et al. 1992; Needleman et al. 1986; Ordway et al. 1991; Piomelli and Greengard 1990;Schwartz et al. 1988; Vijayaraghavan et al. 1995). Although attenuation of I_A by extracellular AA is well known (e.g., Keros and McBain 1997), we present a potent intracellular oxidative modulation of I_A by AA.

METHODS

Primary rat hippocampal cultures were prepared as described (Müller et al. 1997). Currents were recorded at room temperature (22–24°C) from neurons cultured for 2–3 wk in whole cell patch clamp (SEC-05 l, npi electronics; Tamm, Germany) (Misgeld et al. 1989; Müller and Swandulla 1995) and filtered at 1.3 kHz. The internal solution contained (in mM) 120 KCl, 1 $CaCl_2$, 2 $MgCl_2$, 11 EGTA, 10 HEPES, and 20 D-glucose, pH 7.3. For intracellular application, the tip of the pipette was filled with 2–5 μl and backfilled with AA-containing solution. Neurons were continuously superfused (1 ml/min) with external saline containing (in mM) 130 NaCl, 5.4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 25 D-glucose, and 0.001 TTX. Stock solutions of AA and ETYA (100 mM) in DMSO were stored at −18°C. Experimental solutions were prepared immediately before use. DMSO was ineffective. Where indicated, AA or H_2O_2 was added to the extracellular saline immediately before application from a 30% stock. AA, ETYA, glutathione (GSH), and TTX were obtained from Sigma. Averages are given as means ± SE. Unpaired Student's *t*-test was used for statistical analysis.

RESULTS

In whole cell patch-clamp recording, delayed rectifier ($I_{K(V)}$) and A-currents (I_A) were recorded with a 800-ms hyperpolarizing prepulse to −110 mV to remove inactivation of I_A. Hippocampal neurons were depolarized to potentials between −60 and +20 mV in increments of 10 mV. When the prepulse was followed by a 50-ms interval at −45 mV I_A inactivated completely (Connor and Stevens 1971; Klee et al. 1995). By subtracting $I_{K(V)}$ from mixed currents, I_A was isolated. I_A had an amplitude of 0.5–2 nA and decayed with a time constant of 14.8 ± 3.1 ms (Fig. 1*A*, $n = 22$). With intracellular application of AA ($[AA]_i$ 1 pM), I_A started to decrease ≤60 s after obtaining the whole cell configuration to reach a steady state at −54.3 ± 7% within 120–150 s (Fig. 1*B*, $n = 14$). These times were reduced with a 10-fold concentration, but the maximal effect was not enhanced (−52.4 ± 8%, $n = 8$). AA was ineffective on $I_{K(V)}$ (Fig. 1*A*, *inset*) and on the time constant of inactivation of I_A (13.5 ± 4 ms vs. 14.8 ± 3.1 ms in control, $n = 7$).

With extracellular superfusion, even a 100-fold concentration of AA (0.1 nM) was completely ineffective. A 1,000,000-fold concentration effectively suppressed I_A by 56.5 ± 8% ($n = 7$), whereas $I_{K(V)}$ was unaffected (Fig. 1*C*). This insensitivity of I_A to extracellular application and an even slower time course of the effect suggests poor transmembrane diffusion of AA. (Fig. 1, *B* and *D*). I_A and $I_{K(V)}$ remained stable in control recordings (cf. Fig. 1, *B* and *D*).

Activation and steady-state inactivation data were plotted and fitted with the Boltzmann relation $g/g_{max} = 1/[1 + \exp(V_{0.5} - V_m)/S]$ (Fig. 2, control: $V_{0.5a} = -33.9 \pm 3.4$ mV and $S_a = 8.9 \pm 1.0$ mV ($n = 7$) for activation and $V_{0.5\ i} = -74.7 \pm 2.2$ mV and $S_i = -8.22 \pm 0.8$ mV ($n = 5$) for steady-state inactivation. Intracellular application of AA shifted inactivation to the left (1 pM: −3.4 mV, $V_{0.5i} = -78.1 \pm 3.6$ mV, $S_i = -11.1 \pm 0.6$ mV,

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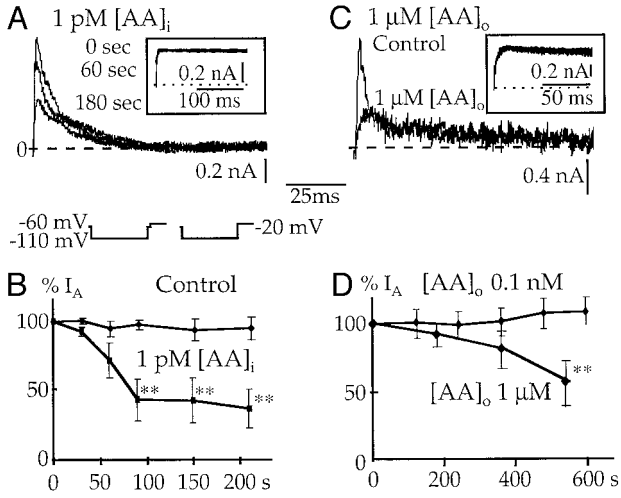

FIG. 1. Suppression of I_A by arachidonic acid (AA). *A* and *B*: intracellular application of 1 pM AA reduces I_A over time (means ± SE, $n = 7$, ** $P < 0.01$) but not the delayed rectifier current ($I_{K(V)}$, *inset*). *C* and *D*: with extracellular application a 1,000,000-fold AA concentration (1 μM) is similar effective, but a 100-fold AA concentration (0.1 nM) is ineffective.

$n = 7$; 10 pM: −9.5 mV, $V_{0.5i} = -84.2 \pm 4.8$ mV, $S_i = -9.4 \pm 1.7$ mV, $n = 7$). This shift can result in significant additional reduction of I_A. Activation was unaffected (1 pM: $V_{0.5a} = -33.6 \pm 2.6$ mV, $S_a = 13.4 \pm 1.6$ mV, $n = 7$; 10 pM: $V_{0.5a} = -36.1 \pm 2.6$ mV, $S_a = 13.5 \pm 2.3$ mV, $n = 7$).

In contrast to AA, eicosatetraynoic acid (ETYA) is neither hydrolyzed by cyclooxygenase nor by lipoxygenase nor by cytochrome P450 (Villarroel and Schwarz 1996). Intracellular ETYA (1 pM) was ineffective in reducing I_A (106 ± 7.3% of control at 150 s, $n = 9$). However, as depicted in Fig. 3*A*, intracellular application of a 100-fold excess of ETYA (100 pM) was similarly effective in suppressing I_A (−36.3 ± 13%, $n = 7$) as 1 pM AA. $I_{K(V)}$ was not affected by ETYA.

AA as well as ETYA are known to break down spontaneously, thereby releasing free radicals that can be inactivated by antioxidants. Figure 3*B* demonstrates that intracellular application of H glutathione (GSH) (2 mM) completely blocked the suppression of I_A by coapplied AA (1 pM). In contrast, extracellular application of GSH (2 mM) did not prevent reduction of I_A by intracellular AA (1 pM).

Ascorbic acid is frequently used to protect AA. In the presence of ascorbic acid (20 μM), either intracellularly or extracellularly, intracellular application of AA was no longer effective in reducing I_A (Fig. 3*C*; intracellular ascorbic acid + AA: 88.1 ± 10.5% at 90–150 s, $n = 7$). To test whether I_A is under tonic oxidative control, we investigated effects of ascorbic acid and GSH on I_A. Figure 3*C* shows that 20 μM ascorbic acid did not enhance I_A by itself. Likewise, intracellular application of GSH (2 mM) did not result in augmentation of I_A (97.8 ± 6.8% of control at 150 s, $n = 9$).

To test oxidative modulation of I_A by a nonlipid oxidant compound, we applied hydrogen peroxide. With extracellular application of 800 μM H_2O_2 for 3 min, I_A was reduced to 64.0 ± 15.5% ($n = 6$). This effect was completely blocked with intracellular application of GSH (2 mM, 100.8 ± 9.7% of control, $n = 6$).

With extracellular application of 1 μM AA, either with or without intracellular application of GSH (2 mM), we observed in two of seven cells an apparently distinct effect reported previously, i.e., an increase in the rate of current inactivation (Keros and McBain 1997).

DISCUSSION

Our experiments demonstrate a novel inhibition of g_{max} of the transient A-current (I_A) by intracellular but not extracellular application of extreme low levels of AA in hippocampal neurons. This suggests limited diffusion of AA across the membrane. A left shift of the voltage dependence of inactivation further reduces I_A depending on membrane potential. The effect of AA does not depend on cyclooxygenase, lipoxygenase, or cytochrome P450 activity but is blocked by antioxidative agents particularly from the inside, most likely caused by an intracellular oxidation of the channel protein. These characteristics discriminate our effect from the highly variable inhibition of I_A and increased rate of current inactivation by extracellular [AA] ≥ 1 μM in the presence of 5 mM [GSH]i (Keros and McBain 1997). Our effect develops significantly faster than the direct effect of AA on expressed Kv 4.2 channels in inside-out patches (Villarroel and Schwarz 1996), suggesting involvement of cytosolic factors in the whole cell configuration. In contrast to inhibition of I_{Ca} by AA (Keyser and Alger 1990), involvement of PKC in inhibition of I_A is unlikely because of the extreme low concentration of AA (1 pM) as well as complete blockade of the effect by antioxidants. We found also no homology of Kv1.4 and Kv 4.2 channel subunits that may underly I_A in our cells with a putative fatty

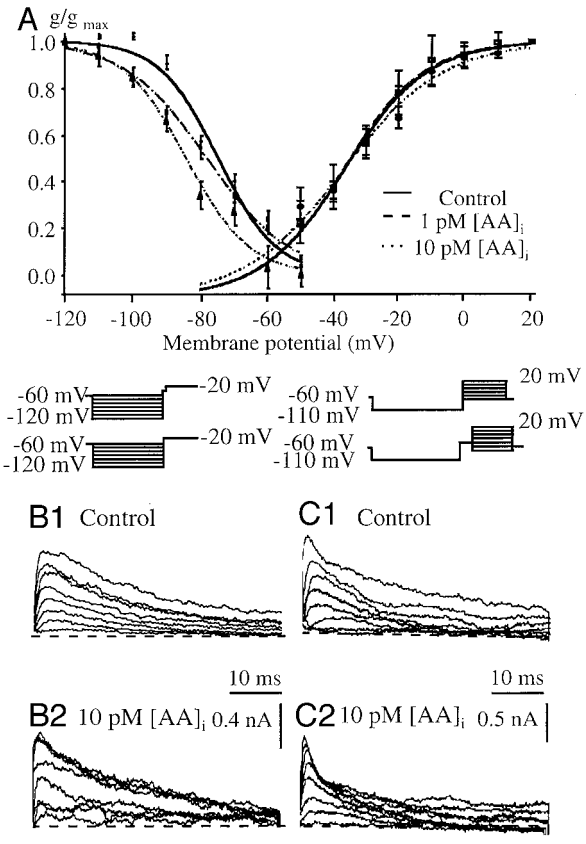

FIG. 2. AA (1–10 pM) shifts steady-state inactivation to the left but does not affect activation of I_A. *A*: conductance-voltage relations and Boltzmann fits ($n = 7$). *B* and *C*: I_A recordings for inactivation (*B*) and activation (*C*).

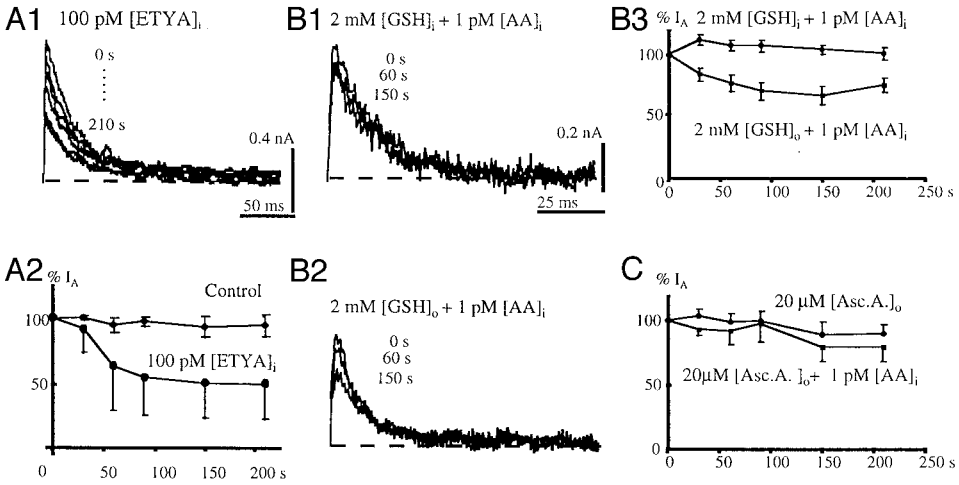

FIG. 3. *A*: at a 100-fold intracellular concentration, the nonmetabolizable AA-analogue ETYA inhibits I_A like AA. *B*: intracellular glutathione (GSH, 2 mM) more effectively blocks inhibition of I_A by AA than extracellular GSH. *C*: acorbic acid, too, blocks inhibition of I_A by AA while not affecting I_A by itself.

acid binding domain in the NMDA receptor or in the rat or human fatty acid binding protein (Petrou et al. 1993).

The effective concentration corresponds to only a few molecules of AA in the cytosol, probably explained by consecutive oxidation of channels by AA continuously diffusing into the cell. The extreme difference between intracellular and extracellular application is amazing for a fatty acid but is in agreement with selective activation of K-channels in cardiac cell membrane by intracellular AA (Kim and Clapham 1989). Either the membrane permeability of AA is low or the membrane passage includes a chemical modification suppressing the effect. Both cases would be of considerable importance for a refinement of the concept of AA as a retrograde messenger. Spontaneous loss of inactivation of Kv1.4-mediated currents by cysteine oxidation is readily reversed by addition of GSH (Ruppersberg et al. 1991). In our experiments I_A was enhanced neither by ascorbic acid nor by GSH. Inhibition of I_A by $[H_2O_2]_o$ requires concentrations of >100 μM, suggesting a specific role for AA.

The extreme sensitivity of I_A to intracellular AA (1 pM as opposed to 1–10 μM) (e.g., Kim and Clapham 1989) suggests a primary physiological role for this mechanism, particularly in conditions of oxidative stress. In perforated patch recordings, glutamate stimulation inhibits I_A PLA_2-dependent in neurons from a mouse model of Down syndrome (unpublished results). In presynaptic terminals, enhancement of glutamate release would have strong implications.

We thank K. Berlin and S. Latta for preparation of cell cultures and Drs. R. Klee and S. Erdmann for helpful discussions.

This work was supported by Deutsche Forschungsgemeinschaft Grant SFB 507/C4, by a Heisenberg stipend to W. Müller, and by Graduiertenkolleg 238.

Address for reprint requests: W. Müller, AG Molekulare Zellphysiologie, Institut für Physiologie der Charité, Tucholskystr. 2, D-10117 Berlin, Germany.

Received 23 December 1998; accepted in final form 1 March 1999.

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