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THE AFTERHYPERPOLARIZING POTENTIAL FOLLOWING A TRAIN OF ACTION POTENTIALS IS SUPPRESSED IN AN ACUTE EPILEPSY MODEL IN THE RAT CORNU AMMONIS 1 AREA

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Abstract—In hippocampal Cornu Ammonis 1 (CA1) neurons, a prolonged depolarization evokes a train of action potentials followed by a prominent afterhyperpolarizing potential (AHP), which critically dampens neuronal excitability. Because it is not known whether epileptiform activity alters the AHP and whether any alteration of the AHP is independent of inhibition, we acutely induced epileptiform activity by bath application of the $GABA_A$ receptor blocker gabazine (5 μM) in the rat hippocampal slice preparation and studied its impact on the AHP using intracellular recordings. Following 10 min of gabazine wash-in, slices started to develop spontaneous epileptiform discharges. This disinhibition was accompanied by a significant shift of the resting membrane potential of CA1 neurons to more depolarized values. Prolonged depolarizations (600 ms) elicited a train of action potentials, the number of which was not different between baseline and gabazine treatment. However, the AHP following the train of action potentials was significantly reduced after 20 min of gabazine treatment. When the induction of epileptiform activity was prevented by co-application of 6-cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX, 10 μM) and D-(−)-2-amino-5-phosphonopentanoic acid (D-AP5, 50 μM) to block α-amino-3-hydroxy-5-methylisoxazolepropionate (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors, respectively, the AHP was preserved despite of $GABA_A$ receptor inhibition suggesting that the epileptiform activity was required to suppress the AHP. Moreover, the AHP was also preserved when the slices were treated with the protein kinase blockers H-9 (100 μM) and H-89 (1 μM). These results demonstrate that the AHP following a train of action potentials is rapidly suppressed by acutely induced epileptiform activity due to a phosphorylation process—presumably involving protein kinase A.

Key words: afterhyperpolarization, SR95531, D-AP5, CNQX, AMPA, NMDA.

Prolonged depolarizations of hippocampal CA1 neurons elicit trains of action potentials that are followed by afterhyperpolarizing potentials (AHPs) of the membrane (Hotson and Prince, 1980; Madison and Nicoll, 1984; Storm, 1989, 1990). By their kinetics, AHPs are classified as fast, medium, and slow. The molecular basis of the medium AHP (mAHP) is thought to involve the small-conductance Ca^{2+}-activated voltage-independent K^+ channel (SK channel) (Stocker et al., 1999; Stackman et al., 2002). Three subunits (SK1–SK3) have been cloned so far in the mammalian brain (Köhler et al., 1996) that are sensitive to the bee venom toxin apamin and the non-peptide blocker UCL1684 (Romey et al., 1984; Blatz and Magleby, 1986; Stocker et al., 1999; Strøbaek et al., 2000; Campos et al., 2000). Recently, we found a striking reduction of UCL1684-sensitive K^+ outward currents in the chronically epileptic rat due to transcriptional down-regulation of SK2 channels causing an impairment of the SK channel-mediated AHP following a series of action potentials (Schulz et al., 2012). Likewise, Behr et al. (2000) have also observed a transient AHP suppression in kindled rats. In the present study, we asked whether the AHP is also affected by non-transcriptional changes. We therefore chose an acute epilepsy model, as, due to the fast time course in this model, transcriptional changes are unlikely to occur, and instead, post-transcriptional alterations could be responsible. Such changes could involve an intermediate phosphorylation of SK2 channels through protein kinase A (Ren et al., 2006; Lin et al., 2008).

Epileptic discharges are commonly induced by the $GABA_A$ receptor blocker bicuculline in the hippocampal slice (Schwartzkroin and Prince, 1980; Williamson and Wheal, 1992). However, the apamin-sensitive AHP has been shown to be depressed by bicuculline (Seutin et al., 1997; Johnson and Seutin, 1997; Debarbieux et al., 1998; Khawaled et al., 1999). Hence, bicuculline appears to be unsuitable to study the AHP in an acute epilepsy model. Because the alternative $GABA_A$ receptor blocker gabazine (also named SR95531) did not exert AHP-blocking actions (Seutin et al., 1997), we chose this compound to acutely induce epileptiform activity in the rat hippocampal slice. We found a significant reduction of the AHP following gabazine treatment. Moreover, this reduction was abolished by exposure to protein kinase inhibitors indicating a phosphorylation process during the establishment of acute epilepsy in the hippocampal slice, and hence a non-transcriptional mechanism underlying the AHP reduction, which appears within half an hour of discharge induction.

EXPERIMENTAL PROCEDURES

Preparation and maintenance of hippocampal slices

All experiments conformed to local (German Animal Welfare Act) and international (European Council Directive 86/609/EEC) guide-

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Abbreviations: CK2, casein kinase II; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione disodium; mAHP, medium afterhyperpolarizing potentials; SK channel, small-conductance Ca^{2+}-activated voltage-independent K^+ channel.

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lines on the ethical use of animals. All efforts were made to minimize animal suffering and to reduce the number of animals used. Adult male Wistar rats (6–8 weeks, Charles River, Sulzfeld, Germany) were anesthetized with S-ketamine (100 mg/kg, i.p.) and xylazine (15 mg/kg, i.p.). Following decapitation, the brain was removed and submerged in ice-cold, sucrose-based dissection solution containing (in mM) NaCl 87, sucrose 75, KCl 2.5, $NaHCO_3$ 25, NaH_2PO_4 1.25, $CaCl_2$ 0.5, $MgCl_2$ 7, and glucose 10, pH 7.4 adjusted with NaOH, osmolarity 310–315 mosmol/kg H_2O. Transversal horizontal brain slices (400 μm) of the hippocampus were prepared using a vibratome (Integraslice 7550 MM, Campden Instruments, Loughborough, UK) and then transferred into a storage chamber containing sucrose-based dissection solution. Slices were continuously gassed with 95% O_2 and 5% CO_2 to maintain the pH at 7.4 and allowed to recover at room temperature for at least 1 h before being used in electrophysiological experiments.

Electrophysiological recordings

For electrophysiological experiments, a hippocampal slice was transferred into an interface chamber (BSC HT, Harvard Apparatus, Holliston, USA) and perfused with standard artificial cerebrospinal fluid (ACSF) containing (in mM) NaCl 124, KCl 3, $NaHCO_3$ 26, NaH_2PO_4 1.25, $CaCl_2$ 2.5, $MgCl_2$ 1.9, and glucose 10, pH 7.4 adjusted with NaOH, osmolarity 303–308 mosmol/kg H_2O (2 ml/min). The recording temperature was maintained at 32 °C (TC-10, npi electronic, Tamm, Germany). Following an equilibration period of 30–45 min, an extracellular borosilicate glass pipette filled with ACSF was placed into CA1 stratum radiatum (2–3 MΩ, fabricated with a vertical puller [PIP5] from HEKA Elektronik, Lambrecht, Germany) to record spontaneous field potentials that were amplified and filtered at 900 Hz (EXT-10-2F, npi electronic) and further processed (Micro1401 and signal 2.16 software, CED, Cambridge Electronic Design, Cambridge, UK).

Intracellular recordings were performed in CA1 pyramidal cells impaled with borosilicate glass microelectrodes (60–130 MΩ, pulled with P-97, Sutter Instrument, Novato, USA and filled with 3 M potassium acetate and 0.3 M KCl) using an SEC-10L amplifier (npi electronic). In these recordings, the resting membrane potential (RMP), the membrane resistance, and the membrane time constant were determined first. The membrane resistance was calculated as the slope of the steady-state current–voltage curve obtained by hyperpolarizing current injection (ranging from −0.2 to −1.4 nA for 600 ms). The membrane time constant was calculated as the average time constant during the hyperpolarizing steps. Subsequent depolarizing current injection (from +0.2 to +1.4 nA) was used to evoke a train of action potentials (600 ms). The AHP was calculated as the difference between the AHP peak amplitude and the RMP (for which the last sweep with +1.4 nA depolarization was used). Because intrinsic firing properties may have an impact on the AHP, only regular spiking CA1 pyramidal cells were included in this study.

Induction of acute epileptiform activity

Acute epileptiform activity was induced by bath application of gabazine (5 μM), which led to spontaneous interictal discharges within 10 min. In a subset of experiments, the induction of epileptiform activity was prevented by co-application of the AMPA receptor blocker 6-cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX, 10 μM) and the NMDA receptor blocker D-(-)-2-amino-5-phosphonopentanoic acid (D-AP5, 50 μM). To study the influence of phosphorylation, protein kinase inhibitors *N*-(2-aminoethyl)-5-isoquinolinesulfonamide dihydrochloride (H-9, 100 μM) or *N*-[2-[[3-(4-bromophenyl)-2-propenyl]amino]ethyl]-5-isoquinolinesulfonamide dihydrochloride (H-89, 1 μM) were co-applied with gabazine. Gabazine, D-AP5, CNQX, H-9, and H-89 were purchased from Tocris Bioscience (Bristol, UK). All other drugs were obtained from Sigma-Aldrich (Taufkirchen, Germany).

Statistical analysis

All data are expressed as mean±standard error of the mean (SEM). The Mann–Whitney U-test was used to determine significance between groups of data. The level of significance is indicated by asterisks (* $P<0.05$, ** $P<0.01$).

RESULTS

Gabazine induces epileptiform activity in the CA1 region

Disinhibition of hippocampal slices by bath application of the $GABA_A$ receptor inhibitor gabazine (5 μM) induced acute epileptiform activity in the CA1 region. As shown in two different slices (Fig. 1A), we performed concomitant field potential (FP) and membrane potential (MP) recordings. After 10-min gabazine treatment, spontaneous fluctuations occurred in the CA1 field potential that were accompanied by membrane potential changes recorded with the sharp microelectrode (Fig. 1A). After 30 min of exposure to gabazine, spontaneous discharges were recorded in the field, and CA1 pyramidal cells exhibited suprathreshold depolarizations with action potentials. As a consequence of the blockade of GABAergic transmission, the RMP shifted toward more depolarized values following gabazine-induced epileptiform activity (baseline: −68.0±1.1 mV, 30 min: −59.5±3.3 mV, $n=14$, $P<0.05$; Fig. 1B). In control experiments without any pharmacological manipulation, we did not observe any significant change of the RMP during the same recording period (baseline: 68.7±1.2 mV, 30 min: 69.1±1.6 mV, $n=8$). When in addition to $GABA_A$ receptor block with gabazine, AMPA and NMDA receptors were blocked by CNQX (10 μM) and D-AP5 (50 μM), respectively, the induction of epileptiform discharges was abolished (data not shown). Interestingly, the RMP remained unaltered also under these conditions (baseline: −70.1±1.1 mV, 30 min: −71.4±1.6 mV, $n=8$; Fig. 1B). Following gabazine treatment, there was also a significant increase in the membrane resistance already present at 10 min of gabazine wash-in (baseline: 45.3±3.5 MΩ, pooled data from 10 and 30 min: 57.2±7.2 MΩ, $n=14$, $P<0.05$; Fig. 1C), which was entirely absent when gabazine, CNQX and D-AP5 were applied together (baseline: 55.2±6.9 MΩ, 30 min: 48.1±3.5 MΩ, $n=8$; Fig. 1C).

Epileptiform activity suppresses the afterhyperpolarizing potential

A prolonged depolarization via current injection of +1.4 nA for 600 ms evoked a train of action potentials, the number of which showed considerable variation among all CA1 pyramidal neurons (14.6±1.2, range 5–36, $n=72$; Table 1). However, there was no significant correlation between the maximum number of action potentials and the afterhyperpolarizing potential ($r^2=0.048$, $n=72$) or the resting membrane potential ($r^2=0.002$, $n=72$), respectively. Hence, we were able to assess the amplitude of the AHP following prolonged depolarization, irrespective of the cell's firing be-