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1-Ethyl-2-benzimidazolinone (EBIO) suppresses epileptiform activity in in vitro hippocampus

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Abstract

Ca^{2+}-activated K^+ currents with medium (mI_{AHP}) and slow (sI_{AHP}) kinetics, that mediate the post-spike medium and slow after-hyperpolarization (AHP), respectively, play critical roles in regulating neuronal excitability and the spread of epileptiform activity and could provide new therapeutic targets for the management of epileptic patients. We tested if the enhancement of the mI_{AHP} by 1-ethyl-2-benzimidazolinone (EBIO) could suppress epileptiform activity in two in vitro models of epileptogenesis induced in CA3 hippocampal pyramidal neurons by superfusion with 4-AP- and kainate-Mg^{2+}-free solutions. Both interictal- and ictal-like epileptiform activities were reversibly suppressed by EBIO concentrations between 200 μM and 1 mM. EBIO predominantly acted by a strong reduction of excitability via an increase (≈450%) of the mI_{AHP}, without changing the sI_{AHP}. Glutamatergic excitatory synaptic transmission was also diminished (≈50%) by 1 mM EBIO. In contrast, EBIO concentrations <400 μM had no effect on synaptic excitation, consistent with a lesser sensitivity to the drug than the mI_{AHP}. Apamine (100 nM), a toxin that specifically inhibits the mI_{AHP}, rapidly and reversibly antagonized the blocking effects of EBIO on epileptiform activity. Our results suggest that manipulations that enhance the mI_{AHP} may prove adequate in the treatment of epilepsies; they also suggest that an abnormal down regulation of the mI_{AHP} may be a key factor in the genesis of hyperexcitable states.

Keywords: Epilepsy; 1-Ethyl-2-benzimidazolinone (EBIO); Calcium activated potassium current; Neuronal excitability; Synaptic transmission; Hippocampal slices

1. Introduction

Epilepsy is a common neurological disorder characterized by an abnormally enhanced electrical activity with synchronous paroxysmal membrane potential oscillations generated by assemblies of neurons. The enhanced electrical activity may result from an imbalance between synaptic excitation and inhibition, and from an increased neuronal excitability (Johnston and Brown, 1981; Prince, 1993; Wong and Miles, 1994; Federico and MacVicar, 1996; reviewed in de Curtis and Avanzini, 2001 and in McCormick and Contreras, 2001). However, the contributions of alterations in intrinsic membrane properties controlling excitability in epileptogenesis are poorly understood.

In pyramidal neurons Ca^{2+}-activated K^+ conductances are particularly important because they supply strong negative feedback control of excitability (Madison and Nicoll, 1984; Borde et al., 2000). Moreover, the Ca^{2+}-activated post-spike after-hyperpolarization (AHP) has been suggested to control epileptogenesis (Matsumoto and Ajmone-Marsan, 1964; Verma-Ahuja et al., 1995; Behr et al., 2000;

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Empson and Jefferys, 2001; Martín et al., 2001). In CA1 pyramidal neurons, Ca^{2+}-activated K^+ voltage-insensitive conductances with medium (≈200 ms) and slow (>2 s) decay kinetics (i.e., the mI_{AHP} and sI_{AHP}, respectively) mediate the medium and slow AHPs, respectively (Sah and Bekkers, 1996; Borde et al., 2000; reviewed in Sah, 1996; Stocker, 2004). In addition, both conductances may be differentiated pharmacologically because the mI_{AHP} is blocked by apamine whereas the sI_{AHP} is insensitive to apamine. The mI_{AHP} and sI_{AHP} provide an efficient negative feedback that counteracts abnormal increases in excitability (Madison et al., 1987; Charpak et al., 1990; Martín et al., 2001). Therefore, drugs that enhance the I_{AHP}s may control abnormal epileptiform activity by reducing excitability.

We examined the effects of 1-ethyl-2-benzimidazolinone (EBIO), a drug that enhances the I_{AHP}s. EBIO was the first benzimidazolinone described as an activator of both of Ca^+-activated K^+ channels and Cl^- secretion (Devor et al., 1996). Because of its effects on Cl^- secretion EBIO was proposed as a potential therapeutic for cystic fibrosis (Devor et al., 1996; Singh et al., 2001). EBIO was also shown to enhance SK channel activity in neurons (Pedarzani et al., 2001; reviewed in Stocker, 2004).

We tested the action of EBIO on the epileptiform activity induced in CA3 pyramidal neurons by challenging the hippocampal slice with 4-aminopyridine (4-AP)+Mg^{2+}-free (Traub et al., 1996; Martín et al., 2001) or kainic acid (KA)+Mg^{2+}-free solutions (Westbrook and Lothman, 1983; Fisher and Alger, 1984; Cherubini et al., 1990). Epileptiform activity was suppressed in a rapid and reversible manner by EBIO. We demonstrate that EBIO acted by reducing the duration of the epileptiform bursts, the number of spikes in the bursts, the burst frequency, the synchronization between bursts in paired recordings, and finally silenced the cells. These effects of EBIO were mediated primarily by a decreased excitability via a specific enhancement of the mI_{AHP} without modification of the sI_{AHP}. These effects of EBIO were reverted by apamine, a specific blocker of the SK channels that mediate the mI_{AHP}. To a lesser extent EBIO also reduced non-NMDA-mediated glutamatergic synaptic excitation.

2. Methods

2.1. Slice preparation

Juvenile Wistar rats (12–17 days) were decapitated and their brains were quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF). The composition of the control ACSF was in mM: 124 NaCl; 2.69 KCl; 1.25 KH_2PO_4; 2 $MgSO_4$; 26 $NaHCO_3$; 2 $CaCl_2$ and 10 glucose. The ACSF was continuously gassed with a 95% O_2–5% CO_2 mixture to attain a pH of 7.3–7.4. Transverse hippocampal slices (400 μm thick) were prepared using a Vibratome (Pleco 101, Series 1000, St. Louis, MO) and incubated more than 1 h in control ACSF in the dark and at room temperature. Slices were transferred to an immersion recording chamber placed on an upright microscope (Olympus BX51WI, Tokyo, Japan) equipped with infrared differential interference contrast (DIC) video microscopy and an ×40 water immersion objective. Slices were maintained at room temperature (22–24 °C) and superfused at a rate of 10 ml/min with gassed ACSF. The exchange of solution in the recording chamber was complete within ≈3 min.

2.2. Electrophysiology

Whole-cell recordings from CA3 hippocampal pyramidal cells were both in the current- and voltage-clamp modes with (4–8 MΩ) patch-pipettes connected to an Axoclamp-2A amplifier (Axon Instruments, Foster City, CA). Pipettes were filled with an internal solution that contained in mM: 135 K^+gluconate, 10 HEPES, 2 ATP, 0.4 GTP, and 1 $MgCl_2$, buffered to pH 7.2–7.3 with KOH. When paired recordings were performed the same methodology was used, except that recordings were also obtained with an EPC-7 amplifier (List-electronic; Darmstadt, Germany). Experiments started after a 15–20 min stabilization period following the establishment of the whole-cell configuration. In voltage-clamp experiments, the holding potential (V_h) was adjusted to −60 or −50 mV. The membrane potential (V_m) was set to the same values (i.e., −60 or −50 mV) in current-clamp experiments by injecting DC current as needed. The m*I*AHP and s*I*AHP were activated under voltage-clamp by a depolarizing voltage command pulse (duration 100 ms, from the V_h to 10 mV). Experiments were rejected if the series resistance changed >20% during recordings. The pClamp 6.0 software (Axon Instruments Inc. Foster Cyty, CA, USA) was used for stimulus generation, data display, acquisition, storage and analysis.

2.3. Stimulation

Synaptic responses were evoked by bipolar mossy fiber (MF) stimulation through a pair of nichrome wires (80 μm diameter), insulated except at the tips, and placed in the stratum lucidum. Electrodes were connected to a stimulator unit (Cibertec, ISU 165, Madrid, Spain) driven by the Clampex program. Stimulation was either with single (0.25 ms; 0.2 Hz) or paired-pulses (50 ms interpulse interval). To isolate excitatory synaptic responses picrotoxin (PTX; 50 μM) was added to block $GABA_A$ mediated synaptic inhibition. The *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) and KA excitatory