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Ictal Activity Induced by Group I Metabotropic Glutamate Receptor Activation and Loss of Afterhyperpolarizations

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Abstract

Exposure to the group I metabotropic glutamate receptor (mGluR) agonist dihydroxyphenylglycine (DHPG) produces long lasting changes in network excitability and epileptiform activity in the CA3 region of rat hippocampal slices that continues in the absence of the agonist and includes both interictal and more prolonged ictal-like activity. We evaluated the afterhyperpolarization (AHP) that follows repetitive neuronal firing in neurons exposed to DHPG and related the change in the AHP to the pattern of epileptiform activity. In contrast to neurons from control slices that had a robust AHP following neuronal depolarization and action potential generation, neurons that had been exposed to DHPG displayed a minimal AHP following depolarization. Whole-cell voltage-clamp recordings showed a small outward or transient inward current following a depolarizing pulse in neurons from slices that had been exposed to DHPG while control neurons had a long lasting outward current. In slices that demonstrated ictal patterns after exposure to DHPG, bath application of 1-ethyl-2-benzimidazolinone (1-EBIO, 1 mM) or 5,6-dichloro-1-ethyl-1,3-dihydro-2H-benzimidazol-2-one (DCEBIO, 100 μM) which enhance the AHP, suppressed ictal discharges. Whole-cell voltage-clamp recordings demonstrated the return of the medium and slow AHP current in neurons that had transiently been exposed to DHPG when 1-EBIO or DCEBIO was bath-applied. Co-application of either 1-EBIO or DCEBIO with DHPG blocked the induction of epileptiform activity. Transient DHPG exposure caused a long-term suppression of the AHP and ictal patterns of epileptiform activity. 1-EBIO or DCEBIO which re-established both the medium and slow AHP suppressed ictal discharges. These results support the hypothesis that the loss of the AHP contributes to the generation of ictal activity after transient DHPG exposure.

Keywords

CA3; Hippocampus; Epilepsy; Apamin; 1-EBIO; DCEBIO

Introduction

During conditions of increased glutamate release such as seizures, group I metabotropic glutamate receptors (mGluRs) activate many second messenger pathways that may contribute to epileptogenesis (Hermanns and Challiss, 2001; Wong et al., 2002). Exposure to the group I mGluR agonist 3,5-dihyroxyphenylglycine (DHPG) results in the induction of epileptiform discharges in hippocampal slices that persist for hours after DHPG is removed (Merlin et al., 1995; Sayin and Rutecki, 2003). The epileptiform activity includes brief interictal discharges and longer (>2s) synchronous activity that resembles ictal activity. In a number of models, the rate of interictal discharges is related to the duration of the afterhyperpolarization that follows

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repetitive firing of CA3 pyramidal neurons (Chamberlin and Dingledine, 1989; Fernández de Sevilla et al., 2006; Lappin et al., 2005; Rutecki, 1995; Rutecki and Yang, 1997). The transition from an interictal to ictal pattern of activity has been hypothesized to occur because of a loss of the afterhyperpolarization (AHP) that follows interictal discharges (Ayala et al., 1973).

Acutely, activation of group I mGluRs leads to a reduction of a number of potassium currents including the currents that mediate the medium and slow AHPs that are activated by intracellular calcium (Mannaioni et al., 2001; Young et al., 2008). Both these AHPs are dependent on increased intracellular calcium, although the medium AHP may also have components that include a M current contribution (Storm, 1989). The calcium dependent component of the medium AHP (mAHP) is mediated by small conducting channels (SK) (Sah and Faber, 2002) and blocked by apamin whereas the channel(s) associated with the slow AHP (sAHP) have not been defined. Recent evidence has shown that exposure to DHPG leads to a long lasting loss of the slow AHP in CA1 and CA3 pyramidal neurons (Ireland et al., 2004; Young et al., 2008). In CA3 neurons the long lasting suppression of the AHP is a result of a reduction of both the medium and slow AHPs and dependent on protein synthesis, p38 MAP kinase, and mGluR5 activation (Young et al., 2008).

We evaluated the AHP that follows neuronal depolarization and action potential generation in CA3 neurons in slices that had been exposed transiently to DHPG. We also investigated the effects of benzimidazolinones (1-ethyl-2-benzimidazolinone [1-EBIO] and 5,6-dichloro-1-ethyl-1,3-dihydro-2H-benzimidazol-2-one [DCEBIO]) that enhance the medium AHP mediated by calcium influx on ictal discharges induced by DHPG exposure (Pedarzani et al., 2005). Our findings demonstrate that the benzimidazolinones reversed the DHPG-induced loss of the slow and medium AHP and suppressed ictal activity. Furthermore the induction of ictal activity by DHPG was inhibited by benzimidazolinones.

Methods

Slice preparation

Following deep anesthesia with pentobarbital (60-75 mg/kg IP) or isoflurane, Sprague-Dawley rats (7-40 d old) were decapitated; the brain was removed and blocked to prepare hippocampal slices using a Lecia vibratome. Iced artificial CSF (aCSF) with elevated magnesium (7 mM $MgCl_2$) and low calcium (0.5 mM $CaCl_2$) was used to bathe the brain during slice preparation. Slices from younger animals (7-21 day old) were used for whole-cell recordings and older animals (28-40 day old) were used for sharp and extracellular electrode recordings. After slices were prepared they were incubated at room temperature in aCSF or aCSF with 3,5-DHPG (100 μM R-S or 50 μM of the S enantomer) for 90-120 min. The aCSF was composed of (in mM) NaCl 124, KCl 5, NaH_2PO_4 1.25, $CaCl_2$ 2, $MgSO_4$ 2, $NaHCO_3$ 26, and glucose 10. Slices were then transferred to an interface chamber and bathed in aCSF for 1 hour before intra and extracellular recordings were made. Slices in the interface chamber were used to characterize the pattern of spontaneously occurring activity in the CA3 region.

For whole-cell voltage-clamp experiments, slices were transferred to a submerged recording chamber for direct visualization using infra-red differential interference contrast for direct visualization of CA3 pyramidal neurons with a CCD camera The aCSF for the whole cell experiments was (in mM) NaCl 130, KCl 3.5, $CaCl_2$ 2.5, $MgCl_2$ 1.5, Glucose 10, $NaHCO_3$ 24, NaH_2PO_4 1.25. In addition 1 μM tetrodotoxin, 10 μM bicculline methiode, and 1 mM tetraethylamonium were added to block voltage dependent sodium channels, $GABA_A$ receptors, and potassium currents including the delayed rectifier, M current, and the fast BK calcium activated current to isolate slow calcium activated potassium currents (Mannaioni, et al. 2001).

Electrophysiological recordings

Extracellular recordings were made from the CA3 region of slices using glass microelectrodes (2-4 MΩ) filled with 150 mM NaCl and the signal amplified with a Getting model 5A amplifier. Spontaneously occurring activity was recorded digitally using Axoscope 8.2 software for at least 5 minutes after placing the electrode. The activity was characterized as no spontaneously occurring burst activity (non-bursting), interictal if only brief (< 500 ms) recurrent synchronous bursting was noted or ictal if synchronous activity was prolonged (>2s) with synchronous bursting at greater than a 2 Hz frequency. In slices that demonstrated ictal patterns, we added 1-EBIO (1 mM) or DCEBIO (100 μM) to the bathing solution and assessed the effect on the ictal pattern. These concentrations have been shown to enhance the medium AHP current by SK channels (Pedarzani et al., 2005). Following 1-EBIO or DCEBIO exposure, we evaluated if apamin (50 nM), a blocker of the mAHP, or isoproterenol (10 μM), which blocks the mAHP and sAHP (Madison and Nicoll, 1986), would then change the pattern of epileptiform activity.

Control slices incubated in ACSF and slices incubated in ACSF and DHPG were used to make sharp electrode intracellular recordings using microelectrodes (30-100 MΩ) filled with either 4 M K acetate or 2 M KCH_3SO_4 and amplified using an Axocclamp 2A amplifier (Axon instruments, Foster City CA). Intracellular recordings were made to evaluate the AHP following depolarizing current pulses (100-200 ms, 0.3-0.5 nA). The input resistance was measured using negative current injection and resulting voltage change, and the membrane potential was corrected for any offset noted after pulling the microelectrode out of the neuron.

Whole-cell recordings were made using an Axoclamp 200B amplifier (Axon instruments, Foster City CA) to characterize the current that follows a depolarization in control CA3 neurons or from neurons in slices that had been exposed to DHPG. The amplified signal was filtered at 2 kHz and digitized at 10 kHz. CA3 neurons were visualized using a Zeiss Axioscope upright microscope fitted with a 40X water immersion objective. The recording pipette contained (in mM) KCH_3SO_4 140, $MgCl_2$ 3, HEPES 10, Na_2 ATP 2, Na_3- GTP 0.4, and phosphocreatine di(tris), 10 (pH 7.2 and osmolality of 290 mOsm) and had a resistance of 5-10 MΩ. The input resistance was continuously monitored throughout the recording period, and recordings were abandoned if the input resistance changed more than 15%. The voltage was stepped from −50 to +70 mV for 200 ms. The peak outward current that followed the depolarizing step was measured and compared between neurons from slices that had been exposed to DHPG and control ACSF exposed neurons.

We also evaluated the effects of 1-EBIO (1 mM) or DCEBIO (100 μM), compounds that enhance the AHP, in control neurons and neurons that had been exposed to DHPG and had a reduced or absent outward current measured following a depolarizing voltage step. After the 1-EBIO or DCEBIO caused an increased outward current that followed the depolarizing step, apamin (50 nM) a medium AHP blocker was added to the bathing saline and the change in peak outward current and area of the outward current were measured.

Induction of epileptiform activity

The effects of 1-EBIO and DCEBIO on DHPG's induction of epileptiform activity were evaluated. Slices were prepared and then either incubated in DHPG alone or with 1-EBIO (1 mM) or DCEBIO (100 μM) and then DHPG. The percentage of slices demonstrating interictal or ictal activity 1 hour after placing in an interface chamber was noted in control DHPG exposed slices and slices that also had been bathed in 1-EBIO or DCEBIO.

Statistics

Comparisons before and after drug exposure were made using Student's paired t-test when normally distributed. Otherwise the Mann Whitney Sum test was used. If multiple comparisons

were made, then an analysis of variance was used. Chi square testing was used to compare the proportion of slices demonstrating different patterns of epileptiform activity. Significance was set at $p < 0.05$ and all values are means ± standard error of the mean (SEM).

Drugs

All drugs were obtained from Sigma except for DHPG which was purchased from Tocris Cookman. DMSO was used as solvent for 1-EBIO and DCEBIO but was less than 1% in the final concentration, a concentration that has no effect on epileptiform activity induced by DHPG.

Results

Loss of the AHP following DHPG exposure

Sharp microelectrode recordings from control CA3 neurons demonstrated a robust afterhyperpolarization following current injection (0.5 nA, Figure 1). On average the peak amplitude of the afterhyperpolarization was −9.4 ± 1.0 mV and the half decay time was 1075 ± 148 ms (n = 21). In slices that had been exposed to DHPG that demonstrated interictal or ictal activity, neurons had a small or no afterhyperpolarization following repetitive firing when compared to control CA3 neurons (Figure 1). Even when the current injection was increased beyond 0.5 nA and more action potentials were produced, a prominent AHP was not observed. For slices that demonstrated ictal patterns the average peak of the AHP was −0.5 ± 0.5 mV with a half decay time of 43.4± 13.7 ms (n = 20). The decrease and brief duration of the half decay time suggested that the slow AHP was more reduced than the medium AHP.

In a limited number of neurons (n = 5) from slices that only demonstrated spontaneously occurring interictal discharges the average AHP amplitude was −2.1± 0.7 mV and half decay time of 62.3 ± 22.5 ms. The slices exposed to DHPG showed significant reductions in both the AHP amplitude and half decay time when compared to controls ($p < 0.001$ ANOVA, Figure 1). There was a trend for the AHP to be larger and longer in interictal slices, but this did not reach statistical significance by post hoc analysis. The differences in the AHP were not associated with differences in the membrane potential or input resistance of neurons from each group (Table 1),

We also evaluated the current underlying the AHP using whole-cell voltage-clamp techniques. In control neurons, a long-lasting outward current followed depolarization from −50 to +70 mV for 200 ms. In neurons from slices that had been exposed transiently to DHPG a small, shorter lasting current was noted. The difference in the peak current between the control and DHPG exposed slices was significant (control 171.3 ± 13.6 pA, n = 24 vs. DHPG-exposed 17.8 ± 3.5, n = 26; $p < 0.001$ Student's t-test). The area of the AHP current was also reduced significantly by prior DHPG exposure (584 vs 15 pA-S median values, $p < 0.001$, Mann-Whitney U statistic). Both the medium and slow AHP currents were reduced after exposure to DHPG (Figure 2).

Loss of ictal pattern with 1-EBIO and DCEBIO

Because of the pronounced loss of the AHP following depolarization in neurons exposed to DHPG, we tested whether the benzimidazolinones compounds, 1-EBIO and DCEBIO, which enhance the medium AHP (Pedarzani et al., 2005) altered the pattern of epileptiform activity induced by DHPG exposure.

1-EBIO (1 mM) was applied to 16 ictal slices and converted the pattern to interictal in 7 slices and stopped spontaneously occurring activity in another 7 slices. Three slices had ictal patterns return after addition of isoproterenol (10 μM), a β adrenergic agonist that reduces both the

medium and slow AHP. Adding apamin (50nM), a potent medium AHP inhibitor, restored ictal patterns in another 4 slices (Figure 3). In control neurons, the peak current associated with the AHP was reduced by nearly 50% by apamin and isoproterenol (132.6 ± 25.8 in control to 70.6 ± 22.3 pA, n = 5, $p < 0.005$) and the area of the AHP current was reduced by nearly 70% (552 ± 133 in control to 170 ± 39 pA-S following isoproterenol and apamin, n =5, $p < 0.02$, data not shown).

Similar results were obtained with DCEBIO (100 μM). In 12 slices that displayed ictal activity following DHPG exposure, DCEBIO converted 9 to an interictal pattern and stopped spontaneously occurring activity in 2. Application of apamin (50 nM) restored ictal patterns in 6 slices (Figure 3). Apamin alone decreased the peak current of the AHP in control neurons by 22% (132.6 ± 25.8 to 103.4 ± 21.8 pA, n = 5, $p = 0.021$) and reduced the area of the current by 25 % (552 ± 133 to 413 ± 122 pA-S, $p = 0.031$).

Enhancement of AHP current after 1-EBIO and DCEBIO

We then evaluated the effects of 1-EBIO or DCEBIO on the current that followed depolarization in CA3 neurons from slices that had been exposed to DHPG. As noted above, there was minimal outward current generated by a voltage step from −50 to +70 mV. In fact, a small inward tail current was also noted in 60% of neurons (Figure 4). When 1-EBIO (1 mM) was applied to neurons that had been exposed to DHPG, a prolonged outward current returned (Figure 4). The current that underlies the medium AHP current was blocked by apamin and the resulting slow AHP current remained (Figure 4). DCEBIO (100 μM) produced a similar effect with both the slow and medium AHP currents returning in neurons exposed to DHPG that initially had a minimal outward current generation produced by depolarization (Figure 4).

1-EBIO or DCEBIO increased significantly the peak and area of the AHP current in neurons exposed to DHPG. The peak current in DHPG exposed neurons treated with 1-EBIO or DCEBIO was still significantly smaller than the peak current from control neurons after DCEBIO application (Figure 4). The area of the current in DHPG-exposed neurons treated with 1-EBIO or DCEBIO was smaller than the area of the current measure for control neurons. (Figure 4); however, although the peak AHP current was less in DHPG exposed neurons in the presence of 1-EBIO or DCEBIO compared to control neurons, the difference was not significant.

Blockade of induction of ictal patterns with 1-EBIO and DCEBIO

We also evaluated whether the actions of 1-EBIO or DCEBIO could prevent or reduce the epileptogenic effects of DHPG. When either 1-EBIO (1 mM) or DCEBIO (100 μM) was added to the incubating saline before DHPG, a significantly reduced percentage of slices demonstrated epileptiform activity in aCSF. In control slices prepared from the same animal as 1-EBIO pretreated slices, nearly 50 % of slices demonstrated ictal activity in aCSF with another 25% displaying interictal discharges and 25 % not bursting (n = 35 slices). 1-EBIO pretreatment significantly reduced the percent of slices that demonstrating ictal activity to 18% with another 29 % demonstrating interictal activity and over 50% not having spontaneously occurring epileptiform activity (n = 38 slices, $p = 0.015$ by Chi square). Pretreatment with DCEBIO (100 μM) had more dramatic effects with none of 27 slices then exposed to DHPG developing spontaneously occurring epileptiform activity.

Discussion

Transient exposure to DHPG results in a long lasting change in CA3 network excitability that is manifest by spontaneously occurring epileptiform activity that mimics both interictal and ictal discharges. A number of second messenger systems are activated by group I mGluRs

(Hermanns and Challiss, 2001; Wong et al., 2002) and may contribute to long lasting changes in membrane and synaptic properties resulting in network synchronization and the emergence of interictal and ictal discharges. One of the long-lasting changes is the loss of the afterhyperpolarization that follows repetitive neuronal firing and calcium influx and checks prolonged high frequency neuronal activity. The afterhyperpolarization includes medium and slow components that both are depressed following DHPG exposure (Ireland et al., 2004; Young et al., 2008). In CA1 neurons, the effect of the AHP suppression is mediated by either mGluR1 or mGluR5 and dependent on tyrosine phosphatase action (Ireland et al., 2004). In CA3 neurons the persistent suppression is dependent on temperature, protein synthesis, p38 MAP kinase activation, and mGluR5 activation (Young et al., 2008).

Our results show that both the medium and slow AHP currents are decreased following transient DHPG exposure at room temperature and measured at room temperature using whole-cell voltage-clamp techniques. Our DHPG exposure was longer than 30 minutes used by others to demonstrate a temperature dependence of the long-lasting AHP suppression (Young et al., 2008). In slices that demonstrated ictal and interictal activity following DHPG exposure, the AHP that followed neuronal firing was markedly reduced compared to control neurons without any difference in average membrane potential or input resistance. The currents that produce the AHP were also markedly suppressed following DHPG exposure.

The afterhyperpolarization that follows neuronal activity has a number of effects that include favoring neuronal burst activity (Fernández de Sevilla et al., 2006), synchronizing network activity, and controlling the rate of interictal discharges (Chamberlin and Dingledine, 1989; Fernández de Sevilla et al., 2006; Lappin et al., 2005; Rutecki, 1995; Rutecki and Yang, 1997). The AHP has also been thought to prevent more prolonged ictal firing and in this study we provide evidence that ictal activity is favored by the reduction of the AHP, both the medium and slow components. The medium AHP is composed of a hyperpolarization that is mediated by opening M channels and by activating the calcium dependent SK channels (Sah and Faber, 2002). In our experiments we measured current in the presence of TEA which blocks current carried by the M channel (Shah et al., 2002). The slow AHP is mediated by a calcium dependent potassium conductance although the actual channel that mediates this afterhyperpolarization is not known (Sah and Faber, 2002; Tzingounis et al., 2007). The slow AHP is mediated by a molecule, hippocalcin, which senses calcium and results in the opening of a potassium channel (Tzingounis et al., 2007).

Our results demonstrate a long-lasting decrease in both the medium and slow AHP and that re-establishing them toward but not above normal values was associated with a change in network epileptiform activity. In slices that demonstrated just interictal activity following exposure to DHPG, we still found a significant reduction in both the medium and slow AHP following neuronal depolarization and action potential generation. This indicates that the suppression of the AHP was not sufficient for the production of ictal activity. This is also the case in slices made epileptic by adding convulsants in which the interictal rate is increased following a reduction in the AHP but the pattern does not become ictal (Rutecki, 1995).

The ictal pattern of activity could be altered by changing the AHP with the benzimidazolinones, apamin, or isoproterenol suggesting that loss of the AHP was necessary to produce ictal activity in slices that had been exposed to DHPG. Apamin reduced the medium AHP peak current in neurons from DHPG-exposed slices in the presence of 1-EBIO by 52% and by 45% for DCEBIO, proportions higher than control neurons in which apamin reduced the peak current by 22%. This correlated with a change in pattern of epileptiform activity that could restore ictal patterns (Figure 3). A cation current activated by DHPG exposure may also be required to produce prolonged ictal activity (Chuang et al., 2000,Bianchi et al., 2009) and we found that 60% of neurons exposed to DHPG had an inward tail current that may be a cationic current

that persists after DHPG exposure. These two changes in intrinsic neuronal firing properties that follow DHPG exposure appear to act synergistically in producing more prolonged synchronization.

Others have shown that reduction in the medium AHP mediated by SK channels and blocked by apamin can enhance epileptiform activity in hippocampal slices produced by 4-aminopyridine (Garduño et al., 2005). Enhancement of the mAHP with 1-EBIO suppressed bursting in the 4-AP model and blocking the slow AHP was associated with less neuronal synchronization during the burst and enhanced the rate of interictal discharges (Fernández de Sevilla et al., 2006). The mechanism for enhancement of the medium AHP by the benzimidazolinones appears to be by shifting the activation of SK channels to lower calcium concentrations (Pedarzani et al., 2005). Others have not found the benzimidazolinones to increase in the slow AHP current in CA3 neurons (Garduño et al., 2005; Pedarzani et al., 2005); however, following suppression of the AHP by DHPG exposure, we found that both 1-EBIO and DCEBIO re-established both the slow and medium AHP current. This finding suggests that the suppression of the slow AHP following DHPG exposure may relate to impaired coupling of calcium concentration and channel activation that is corrected by the benzimidazolinones. Others have shown that DHPG acutely does not reduce the amount of calcium entering CA3 neurons following depolarization (Young et al., 2003) so that the long lasting impairment does not appear to be secondary to less calcium entering a neuron with depolarization.

The slow AHP appears to play a role in timing of interictal bursting and neurotransmitters that inhibit the slow AHP will increase interictal burst frequency produced by elevated extracellular potassium or a GABA blocker (Chamberlin and Dingledine, 1989; Rutecki, 1995; Rutecki and Yang, 1997). Furthermore reduction of the AHP enhances high frequency oscillations produced by elevated potassium in the slice (Dzhala and Staley, 2003). These fast ripples are a marker of epileptiform activity in the slice and of areas of the brain that give rise to seizures.

The medium AHP controls dendritic responses to synaptic input. Deafferentation of CA1 from CA3 input in organotypic hippocampal slices is associated with enhanced dendritic excitability that is mediated by a reduction in SK activity in the dendrites (Cai et al., 2007). This loss may be homeostatic and makes existing afferents more effective in firing the neuron; however, the result is abnormal excitability that may play a role in the development of posttraumatic epilepsy. Activation of SK channels in the dendrites is an important mechanism of controlling postsynaptic excitability and these channels are activated by calcium flux through NMDA channels (Gu et al., 2008). Spontaneously occurring excitatory synaptic potentials are prominent in neurons that had been exposed to DHPG (Figure 1) and re-establishing the mAHP with 1-EBIO and DCEBIO is expected to decrease the decrease the impact of postsynaptic excitatory synaptic activity. Furthermore, the slow AHP helps synchronize the recurrent synaptic activity in the CA3 that leads to interictal bursting (Chamberlin and Dingledine, 1989; Fernández de Sevilla et al., 2006) and its loss may allow for a more prolonged activation of recurrent circuits that underlies ictal activity.

Our results link the loss of the AHP to more prolonged ictal synchronization. The medium and slow AHP are blocked or greatly reduced following DHPG exposure and they return following 1-EBIO or DCEBIO treatment. The loss of the AHP is associated with ictal epileptiform activity and re-establishment of the AHP by 1-EBIO or DCEBIO suppressed epileptiform activity. When the medium AHP is then blocked by apamin, ictal activity returns. We did find that isoproterenol which blocks both the medium and slow AHP (Madison and Nicoll, 1986) also could re-establish ictal bursting in the presence of 1-EBIO. The combined findings support the notion that the reduction of both the medium and slow AHP that persists after DHPG

exposure and that re-establishing them to near normal strength with the benzimidazolinones changes ictal network behavior to either interictal or no spontaneous bursting.

Lastly, the effect of 1-EBIO and DCEBIO in preventing the development of epileptiform activity by DHPG shows that these compounds have not only an anti-epileptic activity but also an anti-epileptogenic effect. We have found that low concentrations of TTX will prevent the induction of epileptiform activity by DHPG (Karr and Rutecki, 2008). The effect of the benzimidazolinones may be caused by a decrease in the firing properties of the CA3 neurons and resultant fewer action potentials generated and less activation of the recurrent synaptic network that is required for the induction of long-lasting network activity changes (Karr and Rutecki, 2008).

In summary, our results demonstrate a correlation of the loss of the medium and slow AHP with ictal activity, and suggest that the AHP contributes to the regulation of network synchrony. Furthermore, re-establishing the AHPs with 1-EBIO or DCEBIO converted ictal activity to interictal or no spontaneous activity supporting the hypothesis that in this model the loss of the AHP is necessary but not sufficient for ictal activity. Initial studies have shown that 1-EBIO does have anticonvulsant effects in vivo, but has toxic side effects that may limit its therapeutic use (Anderson et al., 2006). DCEBIO or other similar compounds have not been studied to see if their therapeutic index is more favorable. Compounds which enhance the AHP may offer a therapy to prevent the transition from an interictal to ictal pattern and also prevent the epileptogenic effects of group I mGluR activation.

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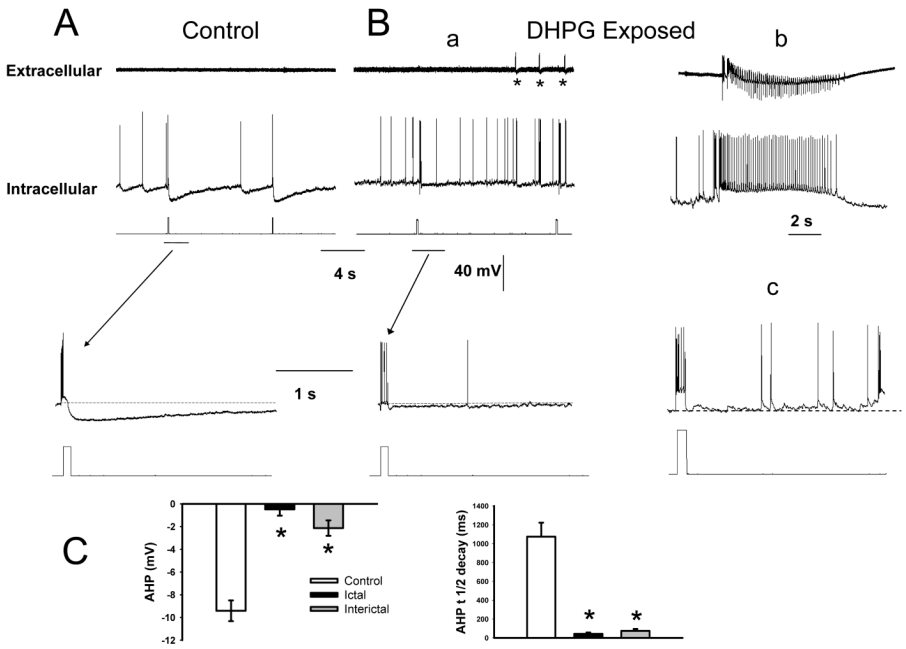

Figure 1.

Neurons from slices exposed to DHPG with spontaneously occurring epileptiform activity had a reduced or absent AHP after depolarization and action potential generation. A: Example of a CA3 neuron from a control slice that showed a robust AHP after a depolarizing current injection (0.5 nA). Spontaneously occurring action potentials also had an associated AHP. Vm = −56 mV. B: Recording from neurons from slices that had been exposed to DHPG that demonstrated interictal (a) and ictal discharges (b). a: A depolarizing current pulse (0.5 nA) produced action potential generation but a small AHP. V_m = −52 mV. The top trace shows an extracellular recording with interictal discharges (*) that are associated with a depolarization and burst of action potentials that is followed by a small afterhyperpolarization. At times, this slice also displayed ictal patterns. b,c: A recording from another neuron from a slice demonstrating ictal patterns after DHPG exposure. Increasing the intensity of the current injection to 1.4 nA produced multiple action potentials but no AHP (c, V_m = −63 mV). Note the spontaneously occurring synaptic potentials and action potentials in the neurons that had been exposed to DHPG. The voltage scale for the extracellular recording is 1.6 mV for traces in A and B(b) and 0.5 mV for B(a). C: Cumulative data showing a significant smaller AHP in neurons from slices demonstrating ictal and interictal activity (* $p < 0.001$ ANOVA). The duration of the AHP measured as half decay time was also significantly shorter in neurons that displayed ictal and interictal discharges (* $p < 0.001$ ANOVA).

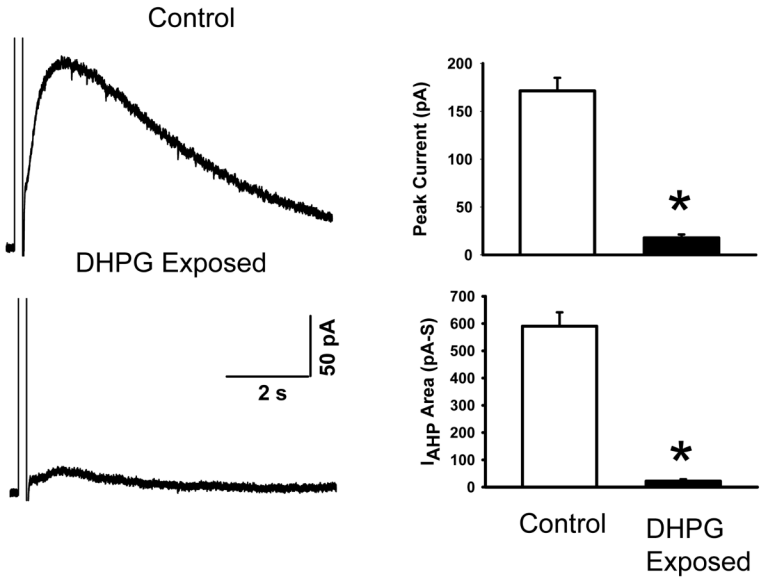

Figure 2.

Prior exposure to DHPG significantly reduced the peak current that follows a 200 ms voltage step from a holding potential of −50 to +70 mV. An outward current that takes time to peak and slowly decays was present in control neurons but was small in neurons that had been transiently exposed to DHPG. Bar graphs demonstrates a significant reduction of the peak AHP current in neurons that had been exposed to DHPG as well as the area of the current (*, $p < 0.001$).

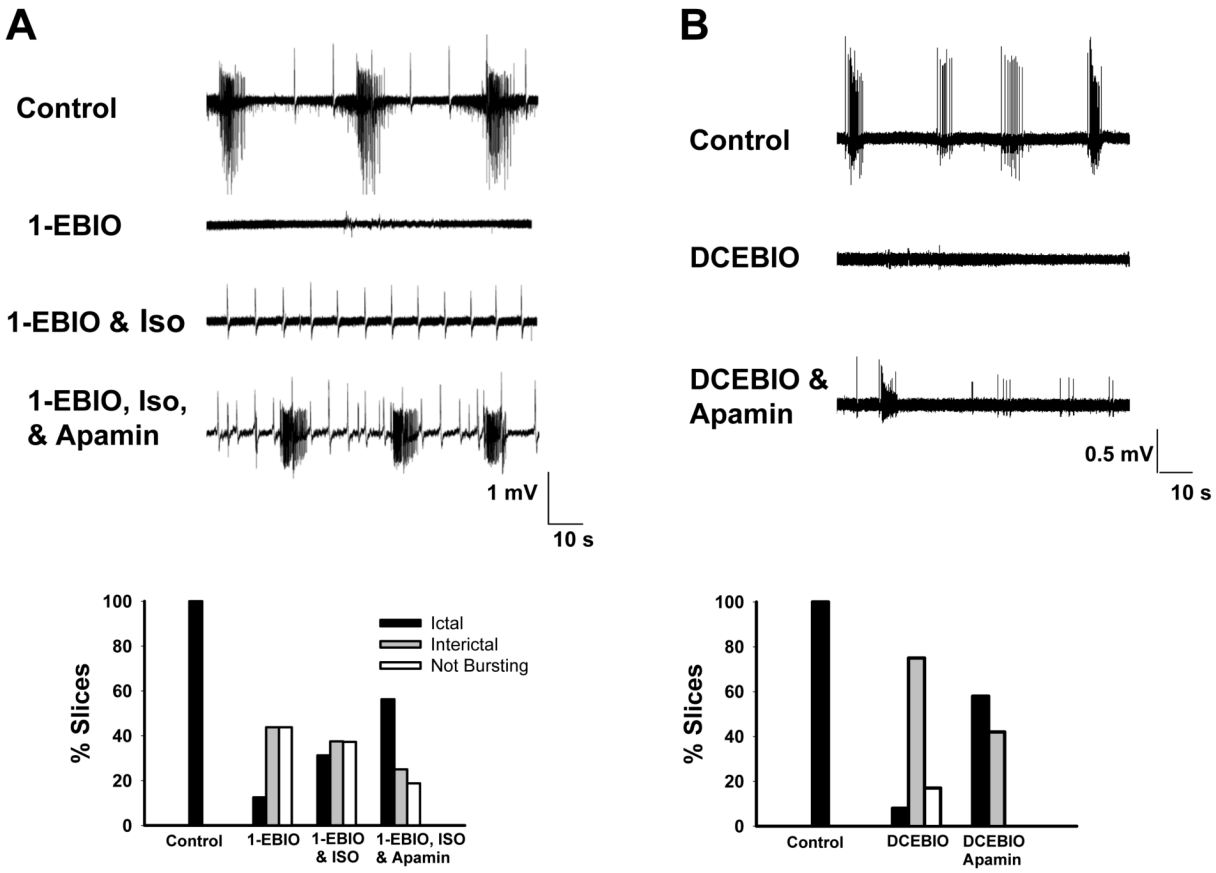

Figure 3.

The benzimidazolinones which enhance the AHP suppressed ictal patterns of activity. A: 1-EBIO (1 mM) stopped ictal epileptiform activity induced by prior DHPG exposure. Extracellular recordings in control aCSF show recurrent prolonged ictal activity that was stopped by 1-EBIO. Co-application of isoproterenol (iso, 10 μM), a beta adrenergic agonist produced an interictal pattern. With further addition of apamin (50 nM), a blocker of the medium AHP, the ictal pattern was re-established. The bar plot shows the effects of manipulations to change the AHP on epileptiform patterns of activity (n = 16 slices). B: DCEBIO (100 μM) produced similar effects in stopping ictal patterns that then could be converted to ictal patterns again with application of apamin (n =12 slices).

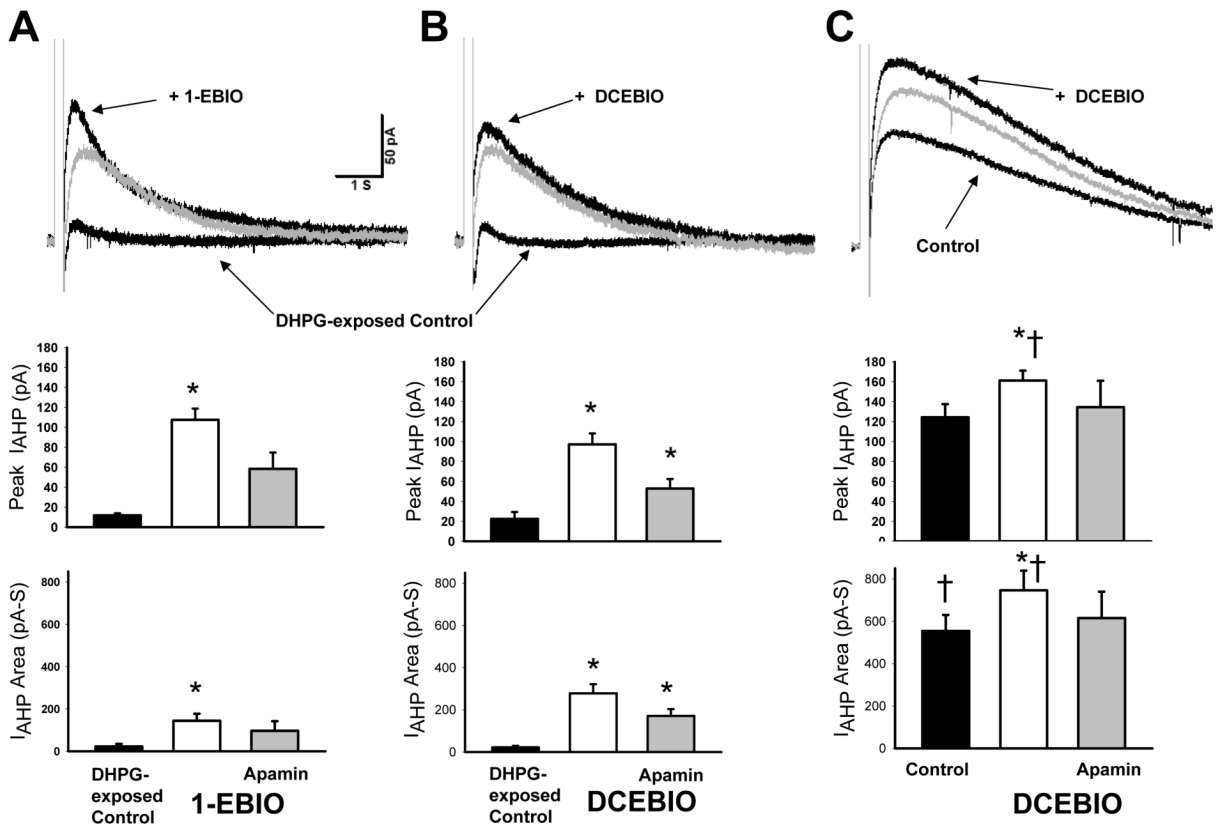

Figure 4.

Benzimidazolinones reversed the loss of outward currents associated with DHPG exposure. A: A depolarizing pulse from −50 to +70 mV produced minimal outward current in a neuron that had been exposed to DHPG. A brief inward tail current was present followed by a small (< 30 pA) outward current. Application of 1-EBIO (1 mM) resulted in a prominent outward current similar to neurons that had not been exposed to DHPG (see C). The early component of the current is reduced by application of apamin (50 nM) which blocks the current associated with the medium AHP but a slow component persists (gray trace). The bar charts shows the peak outward current and area of the outward current in neurons that had been exposed to DHPG, following 1-EBIO application (n = 11), and then the addition of apamin (n =5). The peak amplitude was significantly greater for 1-EBIO compared to control DHPG exposed neurons ($p < 0.001$, ANOVA with $p < 0.05$ for post hoc analysis). For the area of the current, the area in the presence of 1-EBIO was significantly different from control DHPG-exposed neurons (ANOVA, $p = 0.011$, $p < 0.05$ post hoc analysis, *). B: DCEIO (100 μM) had similar effects as 1-EBIO and significantly increased the outward current following membrane depolarization. The bar graphs shows peak outward current and area of the current in control DHPG-exposed neurons, following DCEBIO (n = 16) and following DCEBIO and apamin (n =12). The peak current and area of the current was significantly from control DHPG-exposed neurons for both DCEBIO and DCEBIO with apamin ($p < 0.001$ by ANOVA on ranks, $p < 0.05$ post hoc analysis, *). C: Outward currents from a control neuron and following DCEBIO exposure and DCEBIO with apamin (gray trace). The peak control current was similar to the outward current measured in the neuron depicted in B that had been exposed to DHPG and DCEBIO. DCEBIO caused the control outward current to significantly increase and the peak was reduced by the addition of apamin. The bar graphs plot the peak current and area of the AHP current in control neurons (n = 6 for control and DCEBIO, and 2 for DCEBIO and apamin). DCEBIO produced a significant increase in both the peak (*, $p = 0.002$) and area of

the AHP (*, $p < 0.001$). The control peak current was not significantly different from the peak current for DHPG exposed neurons in the presence of either 1-EBIO or DCEBIO ($P = 0.34$, ANOVA); however the area of the current was significantly greater for control neurons then DHPG exposed neurons in the presence of either 1-EBIO or DCEBIO ($P < 0.001$ with post hoc comparisons of $p < 0.05$, †). Furthermore the peak current of the AHP in control neurons in the presence of DCEBIO was significantly greater than DHPG exposed neurons in the presence of either 1-EBIO or DCEBIO ($p = 0.003$, with post hoc $p < 0.05$, †) as was the area of the current ($p < 0.001$, with post hoc of $p < 0.05$, †).

Table 1

Membrane properties of CA3 neurons from control and DHPG-exposed slices

	n	V_m (mV)	R_{in} (MΩ)
Control	21	−61.1 ± 1.5	40.0 ± 2.9
Interictal	5	−58.6 ± 2.4	43.0 ± 5.6
Ictal	20	−58.9 ± 1.5	47.5 ± 4.7

V_m = resting membrane potential, R_{in} = input resistance. No significant difference by ANOVA.