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Short communication

Long-term increases in BK potassium channel underlie increased action potential firing in dentate granule neurons following pilocarpine-induced status epilepticus in rats

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HIGHLIGHTS

- Dentate gyrus granule cells (GCs) play a critical role in seizure generation.
- Pilocarpine-induced seizure changed intrinsic properties of GCs.
- Paxilline and iberiotoxin reversed pilocarpine-induced changes in intrinsic properties of GCs.

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ABSTRACT

Temporal lobe epilepsy (TLE) is the most common form of acquired epilepsy in adult. Since dentate gyrus granule cells (GCs) play a critical role in hippocampal seizure generation, it is, therefore, important to understand changes in intrinsic properties of GCs in TLE. In this study, the electrophysiological properties of GCs obtained from epileptic rates were compared with the control group using whole cell patch-clamp recording. Results indicated a significant increase in the number of action potentials (APs) in depolarizing currents of 150 pA, 200 pA, and 250 pA. In addition, there was a significant decrease in AP half-width of GCs. The amplitude of fast afterhyperpolarization (fAHP) in epileptic group significantly decreased compared to control group. Blockade of large conductance calcium activated potassium channel (BK), channels with paxilline and iberiotoxin reversed pilocarpine-induced changes in electrophysiological properties of GCs in epileptic group. These results suggest that the BK channel blockers by reversing the firing properties of GCs might have beneficial preventative effects on pilocarpine-induced electrophysiological changes.

Temporal lobe epilepsy (TLE) is the most common form of acquired epilepsy in adult [1]. Pilocarpine injection is one of the most common procedures used for TLE induction in rodents and is associated with recurrent spontaneous seizures. Pilocarpine-induced status epilepticus (SE) initiates a cascade of brain reorganization events termed epileptogenesis lead to acquired epilepsy [2]. Dentate gyrus granule cells (GCs) have long been a focal point for studies on mechanisms responsible for epileptogenesis since they are believed to play a critical role in the generation of hippocampal seizures [3–5]. Mossy fiber sprouting [6] as well as loss of hilar GABAergic interneurons are two important mechanisms that are suggested to contribute to epileptogenesis of dentate gyrus, although they are not alone sufficient to generate chronic epilepsy [7,8]. In recent years, much attention has been focused on changes in the intrinsic properties of neurons as a possible mechanism in epileptogenesis and generation of hippocampal epilepsy. For instance, Brenner et al. reported that β4 subunit of large conductance Ca^{2+} activated K^+ channels (BK channels) reduces excitability of dentate gyrus GCs preventing temporal lobe seizures [9]. Also, Young et al. reported a link between upregulation of inward rectifier K^+ channels in GCs and reduced excitability of these cells 2–8 weeks after

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kainic acid-induced TLE [10]. In another study, early and progressive downregulation of dendritic hyperpolarization-activated cyclic nucleotide-gated (HCN) channel in CA1 hippocampal pyramidal neurons 1 week after pilocarpine injection was associated with increased neuronal excitability [11]. Also, our previous work showed alterations in intrinsic firing properties of GCs in early phase of pilocarpine-induced seizure [12]. Here, we have measured changes in firing properties of dentate gyrus GCs using whole cell patch clamp recordings.

Male Wistar rats (150–230 g) were used and housed under standardized housing conditions with a 12 h light–dark cycle (lights on at 7:00 am) and temperature-controlled ($22 \pm 1\,^{\circ}C$) environment. All experiments were performed in accordance with the National Institutes of Animal Care and use guidelines approved by the Institutional Ethic Committee (IEC) at Shahid Beheshti University of Medical Sciences. In this study, animals were treated with a single dose of pilocarpine (350 mg/kg, i.p.; Sigma–Aldrich Co., St. Louis, USA) 20 min after methyl scopolamin (1 mg/kg, sc) injection for minimizing peripheral effects of pilocarpine. Control groups were treated identically but received saline instead of pilocarpine. Diazepam (4 mg/kg, i.p.) was administered after 3 h to stop SE. Only motor seizures of grade three or greater on the Racine scale [13] were scored. Animals were then observed 6 h/day for the occurrence of chronic spontaneous seizures. For epileptic group, we used rats 30 days after pilocarpine injection because most of the rats showed first seizures 15–30 days (average 24 days) after SE induction. Briefly, the rats were anesthetized with ether and decapitated. The brains were then quickly removed and chilled in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 206 sucrose, 2.8 KCl, 1 $CaCl_2$, 2 $MgSO_4$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 10 D-glucose, and equilibrated to a pH 7.4 (with 95% oxygen and 5% carbon dioxide); the osmolarity of ACSF was adjusted to 295 mOsm. Hippocampal transverse slices were cut into 350–400 μm using a vibroslicer (752 M, Campden Instruments, UK). The slices were incubated in ACSF containing (in mM): 124 NaCl, 2.8 KCl, 2$CaCl_2$, 2 $MgSO_4$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10 D-glucose at pH 7.4 and adjusted to 295 mOsm for 1 h at 32 °C and then, stored at room temperature before being transferred to the recording chamber.

Recordings were made with glass electrodes and were pulled with a two-stage vertical puller (Narishige, Japan) from borosilicate glass capillary. The pipettes had a resistance of 3–6 MΩ and filled with intracellular solution containing (in mM): 140 K-Gluconate, 10 HEPES, 2 $MgCl_2$, 2 Na_2-ATP, 1.1 EGTA, 0.1 $CaCl_2$, and 0.4 Na_2-GTP. The pH of the internal solution was set to 7.3 by KOH, and the osmolarity was adjusted to 300 mOsm. Whole-cell recordings were performed using an Axopatch-200B amplifier (Axon Instruments). Current signals were low-pass filtered at 3 5 kHz and digitized online at 10 kHz and stored on a computer for offline analysis. After seals of more than 1 GΩ resistance were established, the whole-cell configuration was achieved simply by applying a brief suction. Recordings were accepted only if the access resistance was less than 20 MΩ, and if it did not change by 20% during the experiment. In addition, only cells with resting membrane potentials ≥−70 mV and input resistance of >200 MΩ were included in the analysis.

To investigate the electrophysiological properties of GCs in current clamp mode, trains of action potentials (APs) were elicited by 1000 ms depolarizing current pulses ranging 50–250 pA from a holding potential of −75 mV. Characteristics of AP were measured based on the first AP of spike during a 1000 ms depolarizing current injection of 250 pA. Passive and active electrophysiological parameters including resting membrane potential (RMP), input resistance (Rin), number of action potentials, fast afterhyperpolarization (fAHP), and AP half-width were measured. Rin was defined by the steepest slope of the current–voltage (*I–V*) curve based on steady-state responses to hyperpolarizing current pulses

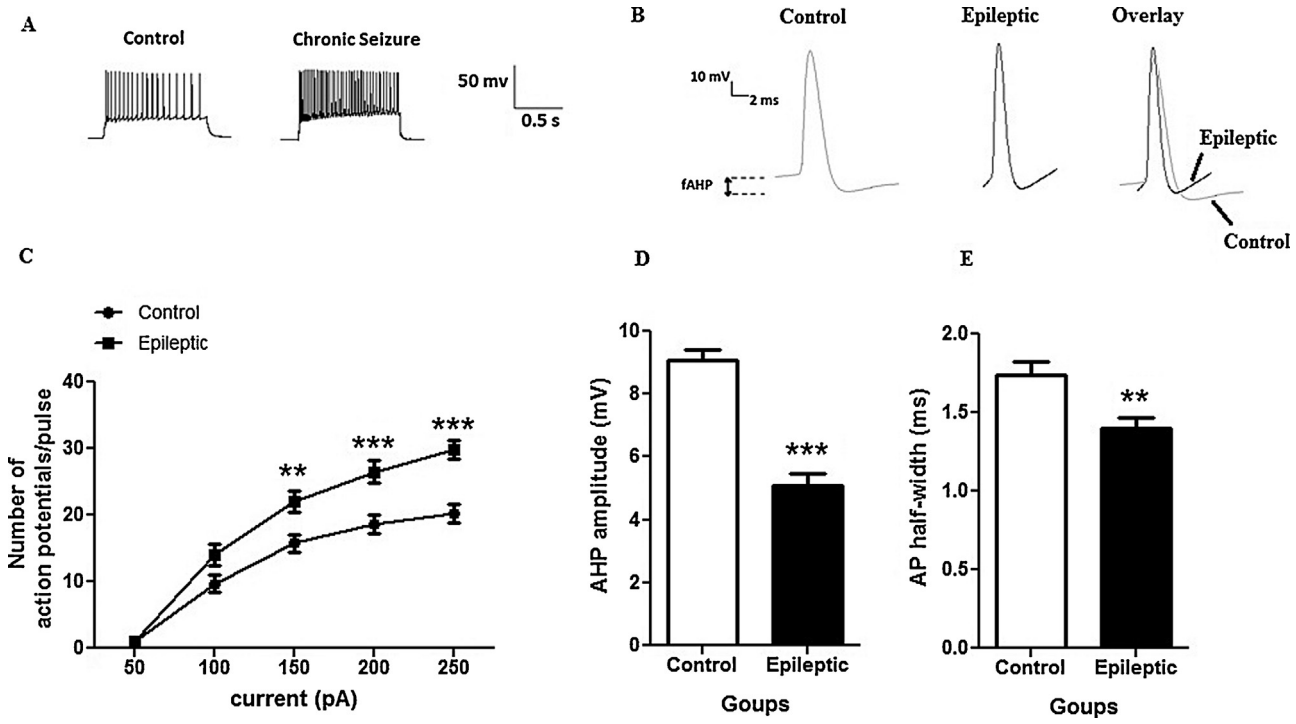

Fig. 1. Changes in the number of action potentials, AHP amplitude, and half-width of action potentials following pilocarpine-induced epilepsy. (A) Representative traces show the differences in firing rate of the GCs in response to 1000 ms depolarizing current injection of 250 pA in control and epileptic groups. (B) Representative trace of first AP of control and epileptic groups during a train of AP evoked by a 250 pA current injection for 1000 ms. (C) Depolarizing current injection ranging from 50 pA to 250 pA increased the number of APs in epileptic group compared to control group. (D) fAHP amplitude significantly decreased in epileptic group compared to control group. (E) AP half-width significantly decreased in epileptic group compared to control group. The results are shown as mean + SEM ($N = 10$ for all groups).
$^{**}P < 0.01$, $^{***}P < 0.001$ significant difference compared to control group (Student's *t*-test and two-way ANOVA followed by Bonferroni's posttest).