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Research Report

Characterizing the persistent CA3 interneuronal spiking activity in elevated extracellular potassium in the young rat hippocampus

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ABSTRACT

Seizures coincide with an increase in extracellular potassium concentrations $[K^+]_e$ yet little information is available regarding this phenomenon on the firing pattern, frequency and neuronal properties of inhibitory neurons responsible for modulating network excitability. Therefore, we investigated the effects of elevating $[K^+]_e$ from 2.5 to 12.5 mM on CA3 rat hippocampal interneurons *in vitro* using whole-cell patch-clamp recordings. We found that the majority of interneurons (21/25) in artificial cerebral spinal fluid (aCSF) exhibited spontaneous tonic spiking activity. As the $[K^+]_e$ increased to 12.5 mM, interneurons exhibited a tonic, irregular, burst firing activity, or a combination of these. The input resistance decreased significantly to 59±18% at 7.5 mM K^+ and did not further change at higher $[K^+]_e$ while the amount of K^+-induced depolarization significantly increased from 5 to 12.5 mM K^+ perfusion; a depolarization block occurred in 4 of the 12 interneurons at 12.5 mM. Also, as $[K^+]_e$ increased, a transition from lower (1.3±0.6 Hz) to higher dominant peak frequency (15.0±5.0 Hz) was observed. We found that non-fast spiking (NFS) interneurons represented the majority of cells recorded and exhibited mostly tonic firing activity in raised K^+. Fast spiking (FS) interneurons predominately had a tonic firing pattern with very few exhibiting bursting activity in elevated K^+. In conclusion, we report that raised $[K^+]_e$ in amounts observed during seizures increases hippocampal CA3 interneuronal activity and suggests that a loss or impairment of inhibitory function may be present during these events.

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Abbreviations: $[K^+]_e$, extracellular potassium concentrations; CA3, cornu ammonis 3; CA1, cornu ammonis 1; aCSF, artificial cerebral spinal fluid; DIC-IR, differential interference contrast-infrared; *I–V*, current–voltage; APs, action potentials; ANOVA, analysis of variance; GHK, Goldman–Hodgkin–Katz; ISI, interspike interval; PDS, paroxysmal depolarization shifts; NFS, non-fast spiking; FS, fast spiking; ADPs, after-depolarization potentials; GABA, gamma aminobutyric acid; NMDA, *N*-methyl-D-aspartate

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1. Introduction

Epilepsy is a common neurological disorder affecting 1–2% of the general population (Browne and Holmes, 2001; McNamara, 1999) and is thought to arise from the hyper-excited, hyper-synchrony of neuronal ensembles (Andersen et al., 1971; Derchansky et al., 2008; Haas and Jefferys, 1984; Velazquez and Carlen, 1999). Epileptic activity has a significant and lasting impact on the brain and on the quality of life for people with this neurological disease.

Although the exact mechanisms underlying seizure generation and initiation have not been elucidated, increased extracellular potassium concentration (K^+_e) is a major manifestation of seizure activity. This phenomenon has been reported in many *in vivo* and *in vitro* studies of seizure occurrence (Benninger et al., 1980; Bikson et al., 2003; Dichter et al., 1972; Fisher et al., 1976; Gutnick et al., 1979; Hablitz and Heinemann, 1987; Heinemann and Gutnick, 1979; Jensen and Yaari, 1997; Krnjevic et al., 1980; Moody et al., 1974; Somjen and Giacchino, 1985; Sykova, 1983; Yaari et al., 1986) which showed that seizure-like activity or paroxysmal after-discharges resulted in an increase in basal levels of $[K^+]_e$ from 2.5–3.0 to 7–15 mM. At present, there is still no consensus regarding the role of raised K^+ on seizure initiation and termination. Some reports support the role of extracellular K^+ in the initiation of hippocampal and neocortical seizures by showing that increase in $[K^+]_e$ during the interictal phase may precede the onset of seizures (Gutnick et al., 1979; Heinemann and Gutnick, 1979) and the addition of raised K^+ solution induces seizure-like events (Izquierdo et al., 1970; O'Connor and Lewis, 1974; Zuckermann and Glaser, 1968). Also, the elevation in $[K^+]_e$ is thought to play a causal role in seizure generation by maintaining seizure recurrence, in a feed-forward fashion, to each recurrent wave of $[K^+]_e$ accumulation via intense neuronal firing (Dichter et al., 1972; Fertziger and Ranck, 1970; Somjen, 1979; Yaari et al., 1986). However, others report that alterations in $[K^+]_e$ is not a causative factor in the initiation or termination of epileptiform activity since changes in extracellular $[K^+]$ did not precede the beginning of each recurrent seizures (Fisher et al., 1976; Lothman et al., 1975; Moody et al., 1974; Stringer and Lothman, 1989). Also, no threshold concentration of extracellular $[K^+]$ has been reported to initiate ictal activity (Gorji et al., 2001; Gorji and Speckmann, 2001). It is believed that the changes in $[K^+]_e$ may be more involved with the spread of epileptic activity rather than its initiation. Regardless of the specific role of extracellular K^+ in seizure initiation and termination, there is little dispute that raised K^+ will affect cellular properties in the hippocampus and thus will, at the very least, modulate seizure properties.

Surprisingly, little information is present regarding increased extracellular K^+-mediated excitability on hippocampal interneurons. McBain (1994) recorded from CA1 inhibitory neurons in the stratum oriens layer, along with CA1 and CA3 pyramidal neurons in rat hippocampal slices perfused with 8.5 mM K^+ aCSF using whole-cell patch-clamp recordings and concluded that the action potential activity of the CA1 interneurons is overridden during raised K^+-induced epileptiform activity. This activity is entrained by interictal events occurring in the CA3 pyramidal neuron population. After the establishment of epileptiform activity, synaptic inhibition onto CA1 pyramidal neurons is severely attenuated, possibly via activity-dependent Cl^- redistribution within CA1 pyramidal neurons after prolonged inhibitory input.

Interneurons from the CA3 region of the hippocampus were examined in this study since CA3 pyramidal neurons have been shown to be involved in the generation and initiation of seizure-like activity (Derchansky et al., 2006; Dzhala and Staley, 2003b; Moddel et al., 2003; Wittner and Miles, 2007). The importance of studying the inhibitory neurons of the hippocampus with raised $[K^+]_e$ is highlighted by the fact that local axo-axonic interneurons synapse onto an average of 686 pyramidal neurons (Buhl et al., 1994) while a single basket interneuron innervates approximately 1500–2000 pyramidal neurons (Sik et al., 1995). Therefore, changes in interneuronal activity, mediated by changes in $[K^+]_e$, can have dramatic and extensive effects on the principle cells of the hippocampus and thus modulate network activity during seizure-like events. For example, at physiological concentrations of extracellular K^+, pyramidal neurons receive intense inhibitory synaptic activity, which controls or gate the spread of excitation throughout the entire hippocampus (McBain and Dingledine, 1992; Miles and Wong, 1987) since inhibitory interneurons mediate both feedforward and feedback inhibition of pyramidal neurons (Lacaille et al., 1987).

Although much information is present describing the transition of CA1 and CA3 pyramidal neurons to ictal or seizure-like events with elevated $[K^+]_e$, no paper to our knowledge, examined what effect incremental elevation of $[K^+]_e$ had on the CA3 stratum oriens inhibitory neurons of the hippocampus. Therefore, in this study, we used intracellular recordings from these cells *in vitro* to evaluate the effects of increasing $[K^+]_e$ concentrations from 2.5 to 12.5 mM, in about 2 mM increments, on neuronal membrane parameters and firing patterns.

2. Results

In total, 68 hippocampal interneurons from the oriens sublayer of the CA3 (a–c) were recorded for this study with an input resistance of 280.6±10.9 mΩ and a resting membrane potential of –58.9±0.8 mV. Initially, interneurons were monitored in aCSF (2.5 mM K^+) for 30 min to validate the stability and viability of the recordings. After 30 min of aCSF perfusion, no significant change in resting membrane potential (+2.8±1.5 mV) or input resistance (+6.9±5.9%) was observed. Immediately after achieving a whole-cell recording, the majority of interneurons (21/25) in aCSF exhibited spontaneous spiking activity. However, four interneurons did not show any spontaneous activity, even after 30 min. As the $[K^+]_e$ increased from 2.5 to 5, 7.5, 8.5, 10 and 12.5 mM, distinct and variable firing patterns were observed (Fig. 1). In some cases, interneurons exhibited a tonic, irregular, or burst firing pattern whereas others showed a mixture of these (Figs. 1 and 6B). Any one specific firing pattern was not exclusively observed in any particular concentration of K^+. When interneurons were exposed to 12.5 mM K^+, regardless of the firing patterns observed, 4 of the 12 interneurons underwent a depolarization block (Fig. 2A) with a resting membrane from –59.3±22.3 to –37.9±4.6 mV and a 32.8±8.3% decrease in input resistance