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Research Report

Differential paired-pulse responses between the CA1 region and the dentate gyrus are related to altered CLC-2 immunoreactivity in the pilocarpine-induced rat epilepsy model

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ABSTRACT

The epileptic hippocampus shows differential paired-pulse responses between the dentate gyrus and the CA1 region. However, little data are available to explain this phenomenon. In the present study, we identified the relationship between regional differences of paired-pulse response and voltage gated Cl^- channel 2 (CLC-2)/vesicular GABA transport (VGAT) expression in a pilocarpine-induced rat model. During epileptogenic periods, paired-pulse inhibitions in the dentate gyrus and the CA1 region were markedly reduced. After recurrent seizure onset, paired-pulse inhibition in the dentate gyrus was markedly enhanced, while that in the CA1 region more reduced. Unlike VGAT, CLC-2 immunoreactivity was markedly reduced in the hippocampus during epileptogenic periods and was re-enhanced only in the dentate gyrus after recurrent seizure onset. Linear regression analysis showed an inverse proportional relationship between alterations in CLC-2 immunoreactivity and changes in normalized population spike amplitude ratio within the CA1 region and the dentate gyrus. Therefore, our findings suggest that the regionally specific alterations in CLC-2 immunoreactivity after SE may determine the properties of paired-pulse responses in the hippocampus of the pilocarpine-induced rat epilepsy model.

1. Introduction

The inhibitory postsynaptic potential (IPSP) elicited by $GABA_A$ receptor is dependent on Cl^- conductive movement, which causes hyperpolarization of the neuronal membrane, and thus drives the membrane potential below its threshold value (Kaila, 1994; Sivilotti and Nistri, 1991; Thompson, 1994). Therefore, increased $[Cl^-]i$ induces diminished $GABA_A$ inhibitory currents (Hochman et al., 1999; Misgeld et al., 1986; Thompson et al., 1988; Thompson and Gahwiler, 1989). Indeed, during the abnormal or normal developmental stage, a higher $[Cl^-]i$ evokes GABA-mediated depolarization (Clayton et al., 1998;

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Jang et al., 2001). One of the candidate mechanisms for modulating neuronal Cl^- homeostasis involves voltage gated Cl^- channels (CLCs), which regulates electrical excitability, the ionic composition of intra-/extracellular compartments, and cell volume. Of these channels, CLC-2 channels strongly rectify in the inward direction, which leads to Cl^- efflux during hyperpolarization (Mladinic et al., 1999).

The epileptic hippocampus shows differential paired-pulse responses between the dentate gyrus and the CA1 region. In the dentate gyrus, paired-pulse inhibition is enhanced (Doherty and Dingledine, 2001; Kobayashi and Buckmaster, 2003; Cohen et al., 2003; Leroy et al., 2004), but in the CA1 region it is markedly reduced (Wu and Leung, 2003; Wozny et al., 2005; Michelson et al., 1989). However, little information is available that explains this phenomenon. Recently, we reported that in seizure prone gerbils (a genetic epilepsy model) CLC-2 expression is up-regulated in the dentate gyrus and that infusion of the potential CLC-2 inhibitor (4,4′-diisothiocyanostibene-2,2′-disulfanic acid, DIDS) reduces fast paired-pulse inhibition in the dentate gyrus. These findings indicate that up-regulated CLC-2 expressions may be a compensatory response of reduced $GABA_A$ receptor-mediated fast postsynaptic inhibitory potential (Kim et al., 2005). Therefore, CLC-2 is possibly involved in the enhanced paired-pulse inhibition of the epileptic dentate gyrus. However, the relationship between CLC-2 expressions and paired-pulse responses in the CA1 region of epileptic animals is unclear. Therefore, in the present study, we sought to (1) examine whether CLC-2 expressions are altered in the rat hippocampus during the epileptogenic period, and (2) identify whether changes in paired-pulse responses in the epileptic hippocampus are associated with altered CLC-2 and vesicular GABA transporter (VGAT) expression.

2. Results

2.1. *Electrophysiology*

An electroencephalogram (EEG) and the characteristic responses of the dentate gyrus and the CA1 region to stimulation are shown in Fig. 1. Control animals showed normal EEG in the dentate gyrus (Fig. 1A1) and strong paired-pulse inhibition of the dentate gyrus (Fig. 1A2) and of the CA1 region (Fig. 1A3). In control animals, the normalized population spike amplitude ratios (2nd population spike amplitude/1st population spike amplitude) were 0.31 in the dentate gyrus and 0.38 in the CA1 region. One day after SE, EEG of the dentate gyrus exhibited the frequent occurrence of interictal spikes and occasionally sustained ictal discharges (Fig. 1B1). During this period, multiple population spikes were detected in responses to both stimuli in the dentate gyrus (Fig. 1B2) and in the CA1 region (Fig. 1B3). In addition, the normalized population amplitude ratios were significantly elevated versus controls ($P<0.01$, Fig. 1E). During the epileptogenic period (1–2 weeks after SE), the dentate gyrus showed normal EEG (Fig. 1C1). However, paired-pulse responses in the dentate gyrus and in the CA1 region were markedly facilitated (normalized population spike amplitude ratios, 0.82 and 0.94, respectively (Figs. 1C2–3 and E). Moreover, after recurrent seizure onset (5 weeks after SE), spontaneous synchronized spiking was detected during EEG observations (Fig. 1D1). Long-lasting ictal discharges were observed intermittently and irregularly at least once during recording periods of 10 min, and paired-pulse responses in the dentate gyrus were to be inhibited (Fig. 1D2), while that in the CA1 region they were maintained in a facilitated state (Fig. 1D3). The normalized population spike amplitude ratios of the dentate gyrus and the CA1 region were 0.02 and 1.24, respectively (Fig. 1E).

2.2. *VGAT immunoreactivities*

Within the hippocampi of control rats, VGAT immunoreactivity was strongly detected in neuropil and in the perikarya of some neurons (Figs. 2A1 and B1). Moreover, these expression patterns and distributions of VGAT in the hippocampi of control animals were in accord with the findings of previous studies (Sperk et al., 2003). One day after SE (Figs. 2A2 and B2), VGAT immunoreactivities were unaltered in the hippocampus as compared to control animals. One to two weeks after SE (Figs. 2A3 and B3), VGAT immunoreactivity was markedly reduced only in the dentate gyrus ($P<0.01$, Fig. 2E). Five weeks after SE (Figs. 2A4 and B4), VGAT immunoreactivity in the granule cell layer, molecular layer, and in the polymorphic layer of the dentate gyrus recovered to the control level.

2.3. *CLC immunoreactivities*

CLC-2 immunoreactivity was apparently observed in the hippocampus proper and in the dentate gyrus of control animals (Figs. 2C1 and D1). Briefly, CLC-2 immunoreactivity was detected in the CA1–3 region and in the granule cell layer of the dentate gyrus. One day following SE (Figs. 2C2 and D2), CLC-2 immunoreactivity was significantly reduced in both Ammon's horns and the dentate gyrus, as compared with control animals ($P<0.01$, Fig. 2E). Moreover, this reduced CLC-2 immunoreactivity was maintained at 1–2 weeks after SE (Figs. 2C3, D3, and E) whereas at 5 weeks after SE (Figs. 2C4 and D4), CLC-2 immunoreactivity was significantly elevated only in the dentate gyrus (particularly in the molecular layer), as compared with control animals ($P<0.05$, Fig. 2E).

Linear regression analysis (Fig. 3) identified an inverse proportionality between alterations in CLC-2 immunoreactivity and changes in normalized population spike amplitude ratio with a linear coefficient of correlation of −0.911 (in the dentate gyrus) and −0.743 (in the CA1 region). No proportional relation was found between VGAT and normalized population spike amplitude ratio (0.044 in the dentate gyrus, 0.021 in the CA1).

3. Discussion

The causes of epileptogenesis are related to a failure of inhibitory control, which include insufficient GABA release, a loss of GABAergic interneurons (De Lanerolle et al., 1989; Obenaus et al., 1993), changes in circuits involving GABAergic interneurons (Sloviter, 1987; Bekenstein and Lothman, 1993), and altered expressions of $GABA_A$ receptors (Buhl et al., 1996; Brooks-Kayal et al., 1998).