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Research Report

Role of gap junctions on synchronization in human neocortical networks

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ABSTRACT

Gap junctions (GJ) have been implicated in the synchronization of epileptiform activities induced by 4-aminopyrine (4AP) in slices from human epileptogenic cortex. Previous evidence implicated glial GJ to govern the frequency of these epileptiform events. The synchrony of these events (evaluated by the phase unlocking index, PUI) in adjacent areas however was attributed to neuronal GJ. In the present study, we have investigated the effects of GAP-134, a recently developed specific activator of glial GJ, on both the PUI and the frequency of the 4AP-induced epileptiform activities in human neocortical slices of temporal lobe epilepsy tissue. To delineate the impact of GJ on spatial spread of synchronous activity we evaluated the effects of carbenoxolone (CBX, a non-selective GJ blocker) on the spread in three axes 1. vertically in a given cortical column, 2. laterally within the deep cortical layers and 3. laterally within the upper cortical layers. GAP-134 slightly increased the frequency of the 4AP-induced spontaneous epileptiform activities while leaving the PUI unaffected. CBX had no effect on the PUI within a cortical column or on the PUI in the deep cortical layers. CBX increased the PUI for long interelectrodes distances in the upper cortical layers. In conclusion we provide new arguments toward the role played by glial GJ to maintain the frequency of spontaneous activities. We show that neuronal GJ control the PUI only in upper cortical layers.

Abbreviations: 4AP, 4-aminopyridine; ACSF, artificial cerebrospinal fluid; CBX, carbenoxolone; Cx, connexin; CNQX, 6-cyano-7-nitro-quinoxaline-2,3-dione disodium salt; CPP, 3,3-(2-carboxypiperazine-4-yl)-propylphosphonate; GABA, γ-amino-butyric acid; GJ, Gap Junction; PUI, phase unlocking index; Tl, time lag; TLE, temporal lobe epilepsy

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1. Introduction

Gap junctions (GJ) attract increasing interest as potential targets to cure different pathologies including neurological disorders, cancer and arrhythmia (Eugenin et al., 2012; Nakase and Naus, 2004; Salameh and Dhein, 2005). The GJ are formed by two hemichannels which dock to each other *via* their extracellular loops to form an intercellular communication channel. Each hemichannel is a pore structure made from 6 protein subunits, the connexins (Cx). A Cx is a protein with 4 transmembrane spanning domains, 2 extracellular and 1 intracellular loops, and with intracellular N and C-terminals. GJ allow the diffusion of molecules of up to 1000 Da, including ions, nutrients, metabolites and second messengers (Bennett and Zukin, 2004; Salameh and Dhein, 2005; Sohl et al., 2005).

The presence of GJ has long been inferred in neocortical neurons, at least early during development, from the dye coupling of pyramidal neurons decreasing drastically towards adulthood (Connors et al., 1983). From a wealth of evidences the role played by GJ during the formation and refinement of neocortical synaptic circuitries was postulated (Rörig and Sutor, 1996). More recently studies of GJ utilized pharmacological tools (Salameh and Dhein, 2005). Neocortical slices obtained from temporal lobe epilepsy (TLE) tissues generate synchronous synaptic activity in the presence of 4-aminopyridine (4AP) (Gigout et al., 2006; Louvel et al., 2001). These events persist during application of glutamate receptor antagonists, hence representing the post-synaptic responses of principal neurons to γ-amino-butyric acid (GABA) released following the synchronous firing of interneurons (Louvel et al., 2001). Blockade of $GABA_B$ receptors by CGP35348 increased the amplitude of these GABA-mediated potentials (Louvel et al., 2001), probably by abolishment of $GABA_B$ mediated control of GABA release (Deisz and Prince, 1989; Sutor and Luhmann, 1998), without modifying their rate of occurrence. These activities are abolished by $GABA_A$ receptors antagonists and reflect likely the depolarizing action of GABA at $GABA_A$ receptors in human TLE, due to impaired chloride homeostasis (Deisz, 1999, 2002; Deisz et al., 2011; Huberfeld et al., 2007; Louvel et al., 2001). Bath application of

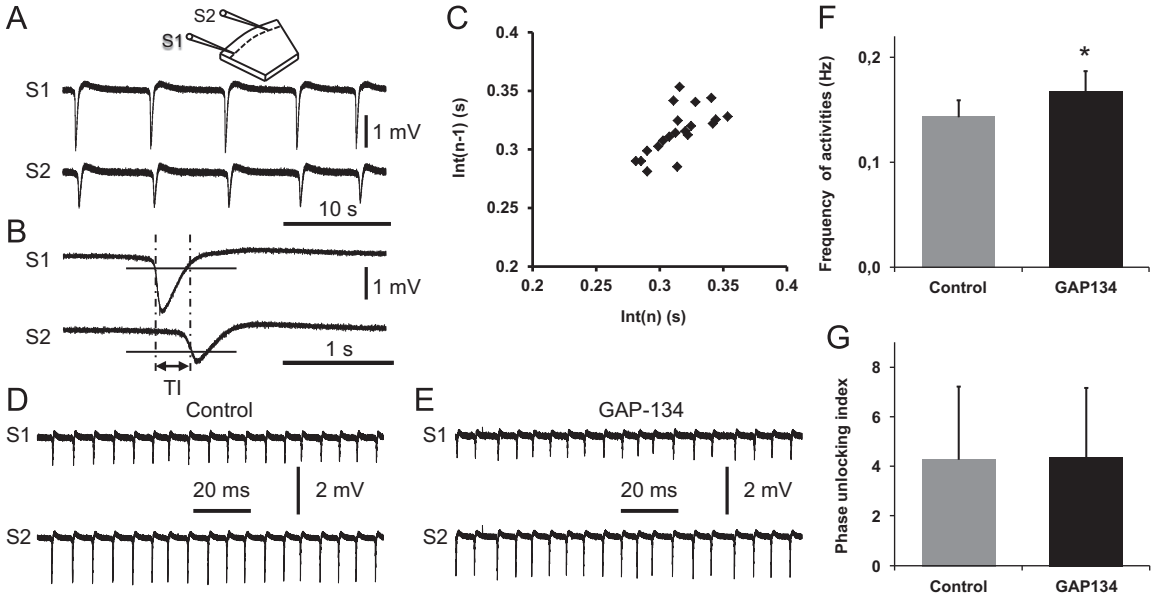

Fig. 1 – Phase unlocking index and effect of GAP-134 on 4-AP induced epileptiform activities. (A) Top: Scheme representing a position of recording electrodes. The electrodes in site 1 (S1) and 2 (S2) are located in the upper cortical layers. Bottom: Spontaneous $GABA_A$ receptor-mediated activities recorded at two sites (S1 and S2) in a human neocortical slice maintained in an interface type recording chamber in the presence of 100 μM 4AP, 10 μM CNQX, and 20 μM DAPV. (B) Expanded traces of spontaneous activities recorded at two sites. The time lag (elapsing between occurrences of activities in two sites of the slice) was measured for each pair of activities as illustrated for one pair and was termed TI (time interval). (C) Scatter plot of time intervals. From the recordings illustrated in A, we measured Tl for 21 consecutive paired activities and constructed a scatter plot TI(n+1)=f(TI(n)). The dispersion in the scatter plot was calculated by summing the distances of all 20 points from the barycentre. This value (expressed in seconds) was termed the phase unlocking index and was calculated for each experimental condition. (D, E) Spontaneous negative GABA-mediated field events in presence of 4AP (100 μM) recorded in control condition (D) and during application of GAP-134 (10 μM). (E) Note that amplitude of the spontaneous activities is not affected and that the frequency is slightly increased. (F) Plot of the frequency of 4AP-induced spontaneous activities in control condition and during addition of GAP-134 (10 μM) as indicated (n=5) in human cortical slices. Note that GAP-134 increased slightly the frequency of the $GABA_A$ receptor-mediated spontaneous activities. *: $p<0.05$ *vs.* control. (G) Plot of the phase unlocking index in control condition and during addition of GAP-134 (10 μM) as indicated (n=5) in human cortical slices. Note that GAP-134 has no effect on the phase unlocking index.