Change in electrophysiological properties of pyramidal cell in animodel of cortical dysplasia
Variation des propriétés électrophysiologiques des cellules pyramidales dans animodel de dysplasie corticale

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Abstract

Rational: Cortical dysplasia has been associated with intractable epilepsy by many clinical studies. Animal model of cortical dysplasia using cortical freeze lesion in early development stage mimic the microgyra described in human.

Methods: Cortical freeze lesion was performed in anesthetised new born rat at postnatal day 1. Whole cell current clamp recordings were used to investigate the changes in electrophysiological properties of CA1 pyramidal cells in this model.

Results: The membrane potential and input resistance of CA1 pyramidal cells were not affected in this model. However the action potential threshold was lowered in lesion group and accompanied by an increase in the firing frequency in response to depolarising currents. In addition, the amplitude of spike afterhyperpolarisations was significantly decreased in lesion group.

Conclusion: The increase excitability of CA1 pyramidal cells in cortical freeze lesion may increase the likelihood to develop epilepsy at adulthood.

Keywords: Hippocampus- Cortical dysplasia- Rat- Patch-clamp- Epilepsy.

Résumé

Rationnel: La dysplasie corticale a été associée à l'épilepsie réfractaire par de nombreuses études cliniques. Le modèle animal de la dysplasie corticale utilisant une lésion corticale au froid durant le premier jour postnatal reproduit la microgyrie décrite chez l'homme.

Méthodes: La lésion corticale au froid a été réalisée sur des rats nouveau-nés anesthésiés. Les enregistrements électrophysiologiques ont été réalisés pour étudier les changements dans les propriétés électriques des cellules pyramidales de la région CA1 de l'hippocampe dans ce modèle.

Résultats: Le potentiel de membrane et la résistance membranaire d'entrée des cellules pyramidales CA1 n'ont pas été affectés dans ce modèle. Cependant, une baisse du seuil du potentiel d'action accompagnée d'une augmentation de la fréquence de décharge en réponse à des courants dépolarisants ont été observés dans le groupe lésion. En outre, l'amplitude des post-hyperpolarisations était significativement diminuée dans le groupe lésion.

Conclusion: L'augmentation de l'excitabilité des cellules pyramidales CA1 après lésion corticale peut contribuer à l'augmentation du risque de développer l'épilepsie à l'âge adulte.

Mots-clés : Hippocampe- Dysplasie corticale- Rat- Patch-clamp- Épilepsie.

Introduction

Abnormal cerebral cortical development is caused by disorder of neural migration during the early developmental stage of the central nervous system. Both genetic factors and extrinsic insults during early life (intrauterine cytomegalovirus infection, hypoxia ischemia, stroke...), may contribute to the development of such cortical malformations [1, 2, 3]. Clinical observations suggest an association between cortical dysplasia and intractable epilepsy, a drug resistant form of epilepsy [2, 4-8]. Studies of cerebral cortex obtained from patients with dysplasia revealed intrinsic epileptogenicity in this area [9, 10].

An animal model of focal cortical malformation have been developed (Dvorak and Feit, 1977) in which cortical freeze lesion early enough in development (postnatal day 0 or 1) mimic the microgyra described in human [11]. Most of the studies using this model have focused on the hyperexcitability [12] reported in the adjacent paramicrogyral zone. Intracellular recordings revealed a change in intrinsic membrane properties of pyramidal cells in the paramicrogyal zone [13], a reduction in GABA-ergic inhibition have been also reported [13, 14-18] in addition to an increase of glutamate-ergic excitation [13, 16, 18, 19].

We have previously shown an increase of both NMDA and non-NMDA mediated excitatory synaptic transmission in CA1 pyramidal cells in cortical freeze lesion rats. Inhibitory synaptic transmission mediated by GABAA receptors was not affected while the one mediated by GABAB receptors was increased. This project will focus on the consequences of cortical freeze lesion on intrinsic membrane properties of CA1 pyramidal cells.

Materials and Methods

Experiments were carried out in Sprague-Dawley rats pups, obtained from Charles River Laboratories (St. Constant, Quebec, Canada) at postnatal day 1 (P1). Pups were kept with their mother in a 12-hour light/dark cycle. The protocol used to induce focal microgyric lesions at P1 has described previously in detail [20, 21]. In brief, newborn rats at P1 were anesthetized with 1 to 3% isoflurane in 100% O2; the skull covering the cortex was exposed. A cooper cylindrical probe (2 mm diameter) cooled with liquid nitrogen is placed for 10 seconds on the skull overlying the right cortex. Naïve control pups were kept away from the dam for an equivalent period of time.

Hippocampal slices were prepared using standard procedures that have been described previously [22] from P16 to P20 rat. Brain was placed in cold artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 2.5 KCl, 1.2 NaH2PO4, 25 NaHCO3, 2.0 CaCl2, 1.0 MgCl2, and 10 dextrose, bubbled with 95% O2/5% CO2, pH 7.3. Hippocampal slices (400-µm thick) were cut with a vibrating blade microtome leica VT1000s (Leica microsystems) and transferred into a container filled with oxygenated ACSF at room temperature. After an incubation period of 1 h, a slice was placed into a recording chamber and maintained submerged using a U-shaped platinum wire. The slice was continuously perfused with oxygenated ACSF at 32°C. Temperature was controlled using CL-100 bipolar temperature controller (Warner instrument).

Hippocampal CA1 cells were visualized with an upright Olympus microscope fit with differential interference contrast (DIC) optics. Recording electrode were folled with a solution contained (in mM): 140 Kgluconate, 5 NaCl, 2 MgCl2, 10 HEPES, 0.5 EGTA, 4.0 Mg2ATP, 0.3 Tris2GTP, 10 phosphocreatine, pH 7.25 adjusted with KOH. Recordings were made using Axopatch 200B (Axon Instruments) with low-pass filtering at 1 kHz. Recordings were digitized and stored with a PC microcomputer-based data acquisition system (Digidata 1200 and pClamp8, Axon Instruments) at 5 kHz.

Results are presented as mean ± SEM, Error bars represent SEM, and the number of cells (n) is given. Statistical differences were computed using ANOVA with Tukey's test for multiple comparisons using Igor (Wavemetrics. Lake Oswego, OR).

Results

Membrane properties of hippocampal CA1 pyramidal cells, in particular the resting membrane potential, action potential threshold, and input resistance, are important factors in determining electrical excitability in response to synaptic inputs. Thus the first component of our study was to characterize changes of pyramidal cells membrane properties, in control and rats with cortical freeze lesion at the first postnatal day (lesion group).

Using whole-cell patch-clamp recordings from hippocampal CA1 pyramidal neurons, we measured the membrane properties of pyramidal cells. Resting membrane potential (Vm), measured as the initial potential recorded after membrane patch rupture, was unchanged (control group -56.5 ± 1.1 mV, n = 10; lesion group -59.3 ± 0.6 mV, n = 10). The membrane input resistance (Rin) was not affect (lesion group, 84.5 ± 4.4 M , n = 10, p = 0.82; control 86.4 ± 6.8 M , n = 9). Action potential threshold was lowered in lesion group (-42.8 ± 1.4 mV n = 10, p=0.01) compared to control (-35.5 ± 0.6 mV, n = 6). We also compared the number of action potentials produced by 250 ms depolarizing current injections. Frequency/current plots showed that the number of spikes evoked by any given current injection was increased in neurons from lesion group when compared with control (Figure 1). The initial firing rate, i.e., the inverse of first inter-spike interval (ISI) duration in response to a depolarizing pulse (300 pA) was significantly increased in lesion group (110.3 ± 13.0 Hz, n = 9, p=0.05) compared to control (69.5 ± 5.9 Hz, n = 6). The initial firing rate was also increased as a function of the injected current. Furthermore, the amplitude of spike afterhyperpolarizations (AHPs) after single action potentials (fast and medium AHPs) were significantly decreased in lesion group (fAHP -6.5 ± 0.7 mV, n = 7, p= 0.01 and mAHP -4.0 ± 0.3 mV, p=0.05) compared to control (fAHP -16.5 ± 3.3 mV and mAHP -11.0 ± 2.5 mV, n = 6).

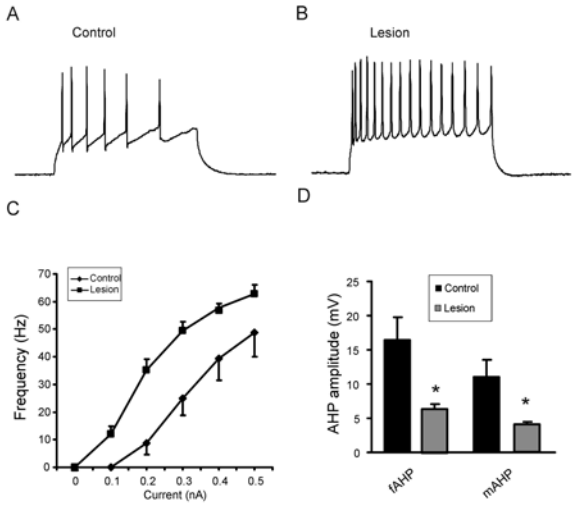

Figure 1: Increase excitability of CA1 pyramidal cells in cortical freeze lesion reflected by an increase of firing frequency and decrease of spike after hyperpolarisations (AHPs) after a single action potential (fast and medium AHPs).

Discussion

In this paper we described a certain number of changes affecting the development of hippocampal CA1 pyramidal cells in response to freeze lesion during the early life of development.

The reduced spike frequency adaptation is presumably the result of a reduction in IAHP channels activity [23] and a lower action potential threshold. AHP are dependent on action potential influx of Ca2+ and activation of Ca2+-activated K+ conductance. The long lasting decrease of IAHP may contribute to an increase of discharge frequency supporting the generation of ictal activity (Matsumoto H et al. 1964). Potential mechanisms for the diminished Ca2+ -dependent AHP include an altered level of calcium-binding proteins, which causes abnormal intracellular calcium regulation, a common feature in epileptic hippocampus [24], or change in Ca2+-activated K+ conductance, or change in voltage dependant Ca2+ calcium channels properties [25].

In conclusion, insults during the early development stage may affect the integrity and function of selected neuronal circuits and may have several neurological consequences including epilepsy.

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