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Short communication

Decrease of neocortical paired-pulse depression in GAERS and possible implication of gap junctions

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HIGHLIGHTS

- Cortical paired-pulse depression is preserved in thalamocortical slice.
- Paired-pulse depression is decreased in GAERS.
- Cortical response to single stimuli is not affected by quinidine and carbenoxolone.
- Paired-pulse ratio is increased by carbenoxolone but not quinidine.
- Glycyrrhizic acid should be considered with caution as control.

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ABSTRACT

Thalamocortical slices are widely used to study thalamocortical relationships and absence epilepsy. However, it is still not known whether (1) intracortical synaptic transmission, in particular neocortical paired-pulse depression (PPD), is maintained in these slices and (2) whether PPD is altered in the Genetic Absence Epilepsy Rat from Strasbourg (GAERS, a model of absence epilepsy for which cortico-thalamic loops are involved). Furthermore, while the involvement of gap junctions (GJ) in the mechanisms leading to epileptiform discharges has been intensively studied, little is known about their effect on intracortical transmission. We first studied intracortical connection efficacy and PPD in thalamocortical slices from GAERS and non-epileptic rats (NER). We then investigated the effects of GJ blockers (carbenoxolone and quinidine) on intracortical response following single or paired-pulse stimulations in coronal slices from Wistar rats. We show that the efficacy of intracortical connections is not impaired in GAERS. We also show that neocortical PPD is preserved in thalamocortical slices of NER, but that its efficacy is strongly decreased in GAERS. Moreover, a NMDA antagonist strongly reduced the PPD in NER but had no effect in GAERS. Cortical responses to white matter stimulation were not modified by quinidine or carbenoxolone in coronal slices of Wistar rats. PPD was recorded in these slices and was decreased by carbenoxolone but not by quinidine. We hypothesize that the decrease of PPD observed in GAERS might be due to a decrease in function of (1) NMDA receptors and/or (2) astrocytic GJ's.

1. Introduction

While thalamocortical slices are widely used to study thalamocortical relationships [1,8,16–18,22,27,33], it is still not known whether intracortical transmission, and in particular neocortical paired-pulse depression (PPD), is maintained in these slices. These aspects are crucial issues for the study of absence epilepsy, for which recurrent activities in corticothalamic loops are involved. Indeed, neocortical PPD is decreased in coronal slices [24] of WAG/Rij rats [35], (a model of absence epilepsy), however, we have previously found in another model of absence epilepsy, the Genetic Absence Epilepsy Rat from Strasbourg (GAERS) [9], that

Abbreviations: ACSF, artificial cerebrospinal fluid; CBX, carbenoxolone; CNQX, 6cyano-7-nitro-quinoxaline-2,3-dione disodium salt; CPP, 3,3-(2-carboxypiperazine-4-yl)-propylphosphonate; GAERS, Genetic Absence Epilepsy Rat from Strasbourg; GJ, gap junction; GZA, glycyrrhizic acid; NER, non-epileptic rat; PPD, paired-pulse depression; PPR, paired-pulse ratio; Quin, quinidine; WM, white matter.

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cortical responses to thalamic stimulations were characterized by short term facilitation in thalamocortical slices [17]. This observation was recently confirmed in a new mouse model of absence epilepsy [18]. In the present study, we have investigated (1) the efficacy of intracortical connections, (2) whether neocortical PPD is preserved in thalamocortical slices of non-epileptic rats (NER, a control strain, free of spike-wave discharges, selected from a Wistar rat colony [9]), and (3) whether PPD is altered in GAERS.

Intercellular communication mediated by gap junctions (GJ) is considered an important mechanism of neuronal synchronization under physiological and pathological conditions such as epileptic seizures [6,28]. For example, previous studies have demonstrated an *in vivo* antiepileptic effect of carbenoxolone (CBX), a GJ blocker, in models of absence epilepsy [13,16]. GJ's are formed by two hemichannels constituted by molecules named connexins [36]. The GABAergic interneurones in the neocortex are interconnected *via* GJ's [12,14] and express neuronal connexin36 [3,23,30]. The role of GJ's in the generation of epileptiform activities, or on cellular excitability, has been intensively studied [5,15,16,32] but few studies focused on their implication in the control of synaptic transmission [16,39]. In the present study, we tested whether GJ's can modulate neocortical PPD in coronal cortical slices from Wistar rats, to study whether the antiepileptic effect of CBX could be mediated by a modulation of neocortical PPD.

2. Material and methods

2.1. Slice preparation

Experiments were carried out on NER ($n = 16$), GAERS ($n = 12$), and Wistar rats ($n = 14$). Since spike-wave discharges in GAERS develop approximately 30 days after birth, to attain a stable frequency of occurrence after 2 months [9], we performed experiments with rats aged 2–3 months. Animals were housed under controlled standard conditions (light/dark cycle, 7:00 AM–7:00 PM lights on) with food and water available *ad libitum*. All animal experiments were carried out in accordance with directive 2010/63/EU and agreed by the Ethics Committee of the "Centre Paul Broca" and the "Bureau de l'Experimentation Animale-INSERM". Adequate measures were taken to minimise pain or discomfort.

Rats were decapitated under deep anesthesia (isoflurane). Brains were quickly excised, placed in cold oxygenated artificial cerebrospinal fluid (ACSF). We cut coronal and thalamocortical slices (500 μm thickness), containing the somatosensory cortex, with a vibratome (Campden Instruments). Thalamocortical slices were obtained using procedures previously described [1,16,18]. The block of tissue containing both somatosensory cortex and thalamus were glued with cyanoacrylate to the vibratome stage, submerged in cold oxygenated ACSF and cut into slices.

Slices were transferred to an interface-type tissue chamber at the interface between oxygenated ACSF and humidified gas (95%O_2/5%CO_2) at 32–35 °C (pH 7.4). The ACSF composition was (in mM): NaCl 124, KCl 3, NaH_2PO_4 1.25, $MgCl_2$ 1.5, $CaCl_2$ 1.5, $NaHCO_3$ 26, and glucose 10.

2.2. Stimulations and responses analysis

Extracellular field potentials were recorded with ACSF-filled electrodes. Signals were fed to high-impedance pre-amplifiers (WPI M-707) and processed through second-stage amplifiers with filtering capability (analogical filters Neurolog; DC-3 kHz). Recordings were digitized (5 kHz) (interface 1401 Plus, C.E.D., UK), stored and analyzed off-line (Spike 2, C.E.D, UK).

After an incubation period of at least 2 h, responses were recorded in cortical layers II/III following electrical stimulation of the underlying white matter (WM) for both coronal and thalamocortical slices.

Constant current stimulations (80 μs; 0–1 mA) were delivered through bipolar stainless steel electrodes. A stimulation of 1 mA enabled a maximal response, with a 10-s interval between single stimuli. We first determined the threshold intensity and increased the stimulus intensity until the amplitude of the synaptic component reached a plateau. The area under the curve of the fiber and synaptic responses were measured at each stimulation intensity and normalized to the maximal response. Linear regression, in the initial linear range, of each curve was used to calculate the slope. This input–output index was named connection efficacy [18].

A response to a single-pulse WM stimulation was composed of (1) an early fiber response and (2) a late excitatory synaptic response, which partially overlapped. To distinguish between these two components CPP and CNQX (antagonists of the excitatory glutamatergic transmission) were applied to the bath *via* perfusion. The remaining response corresponded to the initial fiber component. To isolate the synaptic component we subtracted the initial fiber component from the control response (Fig. 2A).

The paired-pulse ratio (PPR) (defined as the ratio: amplitude of the second synaptic response divided by the amplitude of the first synaptic response) was used to determine the depression/facilitation of the response. In addition, the PPR was calculated for several interstimulus intervals (ISI: 5–100 ms) [18]. The interval between paired stimuli was 30 s.

Because the PPR is strongly dependent on the stimulus intensity, the strength of the electrical stimuli was the same for each slice and adjusted to low (0.1 mA) or high (1 mA) intensity. Both stimulations of the paired stimuli were identical in duration (80 μs) and intensity.

2.3. Solutions and substances

The following drugs were used: 3β-hydroxy-11-oxoolean-12-en-30-oic acid 3-hemisuccinate disodium (carbenoxolone, CBX, 100 μM; Sigma); 6-cyano-7-nitroquinoxaline-2,3-dione disodium salt (CNQX, 10 μM; Tocris); 3-((R)-2-carboxypiperazine-4-yl)-propyl-1-phosphonic acid (CPP, 5 μM, Tocris); glycyrrhizic acid ammonium salt (GZA, 300 μM, Sigma); quinidine hydrochloride monohydrate (Quin, 100 μM, Sigma). All drugs were dissolved in water, prepared as stock solutions and added to ACSF prior to bath-application. Chemicals for ACSF were acquired from Sigma.

2.4. Analysis

Each value is presented as means plus/minus standard error of the mean. The n values correspond to the number of slices. For all comparisons we used a paired or unpaired bilateral t-test and significance was considered if $P \leq 0.05$.

To test the influence of the strain (or drug) on the PPR at several ISI, we performed a 2-way repeated measures analysis of variance (ANOVA) with (1) strain or drug: between-subject factors and (2) ISI: within-subject factor.

3. Results

3.1. Intracortical response in thalamocortical slices of GAERS and NER

3.1.1. Single-pulse stimulation

In thalamocortical slices the neocortical response to single-pulse WM stimulations were composed of two components (1) an early negative fiber component (N1) that persisted in CPP + CNQX followed by (2) a later negative synaptic component (N2) that decreased or disappeared in CPP + CNQX condition. N1 could be