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Molecular and neurochemical substrates of the audiogenic seizure strains: The GASH:Sal model

Ana I. Prieto-Martín [a], J. Daniel Aroca-Aguilar [b], Francisco Sánchez-Sánchez [b], Luis J. Muñoz [c], Dolores E. López [c], Julio Escribano [b], Carlos de Cabo [a,*]

[a] Research Department, Neuropsychopharmacology Unit, Albacete General Hospital, 37 Hermanos Falcó Street, Albacete E-02006, Spain
[b] Department of Genetics, Faculty of Medicine, University of Castilla-La Mancha, 14 Almansa Street, Albacete E-02006, Spain
[c] INCYL, University of Salamanca, 1 Pintor Gallego Street, Salamanca E-37007, Spain

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ABSTRACT

Purpose: Animal models of audiogenic epilepsy are useful tools to understand the mechanisms underlying human reflex epilepsies. There is accumulating evidence regarding behavioral, anatomical, electrophysiological, and genetic substrates of audiogenic seizure strains, but there are still aspects concerning their neurochemical basis that remain to be elucidated. Previous studies have shown the involved of γ-amino butyric acid (GABA) in audiogenic seizures. The aim of our research was to clarify the role of the GABAergic system in the generation of epileptic seizures in the genetic audiogenic seizure-prone hamster (GASH:Sal) strain.
Material and methods: We studied the K^+/Cl^- cotransporter KCC2 and β2-GABAA-type receptor (GABAAR) and β3-GABAAR subunit expressions in the GASH:Sal both at rest and after repeated sound-induced seizures in different brain regions using the Western blot technique. We also sequenced the coding region for the KCC2 gene both in wild- type and GASH:Sal hamsters.
Results: Lower expression of KCC2 protein was found in GASH:Sal when compared with controls at rest in several brain areas: hippocampus, cortex, cerebellum, hypothalamus, pons–medulla, and mesencephalon. Repeated induction of seizures caused a decrease in KCC2 protein content in the inferior colliculus and hippocampus and an increase in the pons–medulla. When compared to controls, the basal β_2-$GABA_AR$ subunit in the GASH:Sal was overexpressed in the inferior colliculus, rest of the mesencephalon, and cerebellum, whereas basal β_3 subunit levels were lower in the inferior colliculus and rest of the mesencephalon. Repeated seizures increased β2 both in the inferior colliculus and in the hypothalamus and β_3 in the hypothalamus. No differences in the KCC2 gene-coding region were found between GASH:Sal and wild-type hamsters.
Conclusions: These data indicate that GABAergic system functioning is impaired in the GASH:Sal strain, and repeated seizures seem to aggravate this dysfunction. These results have potential clinical relevance and support the validity of employing the GASH:Sal strain as a model to study the neurochemistry of genetic reflex epilepsy.

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1. Introduction

The involvement of the GABAergic, glutamatergic, and monoaminergic systems in audiogenic seizures affecting diverse brain regions — largely the inferior colliculus (IC), as well as the superior colliculus (SCol), periaqueductal gray, reticular formation, substantia nigra, amygdala (AMYG), cortex (CTX), hippocampus (HIPP), and even the hypothalamus (HYP) — has been extensively studied and reviewed by Cairasco's, Faingold's, and Coleman's laboratories in audiogenic epilepsy strains [1–7]. Alterations in the GABAergic system seem to be a main feature in the neurochemical mechanisms underlying audiogenic epilepsy. Lower GABA binding sites were found in whole brain homogenates from seizure susceptible DBA/2 mice [8]. Seizure-prone BALB/c mice showed reduced striatal GABA, when compared with seizure-resistant mice [9]. In sublines of inbred Rb mice, susceptibility to audiogenic seizures has been correlated with low levels of GABA in the HIPP, whereas lower amounts of GABA in the olfactory bulb were found to be associated either with seizure-severity or seizure-diversity [10]. Also, the audiogenic absence-like seizures in the C57Bl10 sps/sps mutant have been shown to be associated with lower glutamic acid

* Corresponding author at: Complejo Hospitalario Universitario de Albacete, Unidad de Neuropsicofarmacología, Edificio de Investigación, 3 planta, c/ Hermanos Falcó, 37, E-02006, Albacete, Spain. Tel.: +34 967 597500; fax: +34 967 243952.
E-mail addresses: anaprima05@hotmail.com (A.I. Prieto-Martín), JoseDaniel.Aroca@uclm.es (J.D. Aroca-Aguilar), Francisco.SSanchez@uclm.es (F. Sánchez-Sánchez), lmp@usal.es (L.J. Muñoz), lopezde@usal.es (D.E. López), julio.escribano@uclm.es (J. Escribano), carlosd@sescam.jccm.es (C. de Cabo).

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decarboxylase activity in the CTX and HIPP. Gamma-amino butyric acid binding sites are reduced in these areas, as well as in the mesencephalon (MES), cerebellum (CER), and pons–medulla (P - M) when compared with the parent strain [11]. Genetically epilepsy-prone GEPR-9s rats, however, have been demonstrated to present increased number of GAD-expressing neurons in the IC [12] as well as higher maximal ligand binding for both high and low affinity GABA sites compared to Sprague–Dawley rats that do not display audiogenic seizures [13]. The genetically epilepsy-prone hamster GPG/Vall, which exhibits generalized tonic–clonic seizures in response to sound stimulation was found to have increased levels of GABA in the SCol [14] but lower GABA concentrations in the IC [15] when compared with wild-type Syrian hamsters.

Gamma-amino butyric acid is one of the main inhibitory neurotransmitters in the adult vertebrate central nervous system. Its fast inhibitory actions take place when GABA gates postsynaptic $GABA_A$ ionotropic receptors ($GABA_ARs$). The native $GABA_AR$ is a heteropentamer typically comprised of two α (α_{1-6}), two β (β_{1-3}), and one γ (γ_{1-3}) subunit(s) that form a selective channel for chloride (Cl^-) ions [16]. Under standard circumstances, the Cl^- concentration inside the adult neuron ($[Cl^-]_i$) is lower than in the intercellular space, and the opening of the $GABA_AR$ Cl^- channel after GABA binding produces an influx of Cl^- that leads to hyperpolarization of the cell membrane [17]. Lower Cl^- levels are maintained through the actions of the KCC2 K^+/Cl^- cotransporter that extrudes Cl^- from the neuron [18]. However, during development or in pathological situations, $[Cl^-]_i$ are increased, and $GABA_AR$ actions are depolarizing. This increase in $[Cl^-]_i$ has been associated with lower KCC2 expression both during development and in pathological conditions, including epilepsy [19–23]. Conversely, KCC2 mutations that reduce neuronal Cl^- extrusion have been found to be associated with epileptic syndromes in humans [24,25]. $GABA_AR$ subunit composition determines its functionality [16,26], and it has also been shown to change during development and across brain regions [27–29]. γ-amino butyric acid receptor β2-subunit is one of the most abundant $GABA_AR$ subunits in the whole brain [30], while β_3 is the most abundant β-type subunit in the auditory pathway [29]. Both contain the main binding sites for benzodiazepines [16,30]. They often display complementary distribution and abundance across brain regions [27–29], and alterations in their expression have been widely observed in both human patients [31,32] and animal models of epilepsy [33–35].

The GASH:Sal hamster represents a novel model to study audiogenic epilepsy [36,37]. Previous published reports from our team have demonstrated the epileptic nature of their ictal electroencephalogram [38] and the similarity of the behavioral pattern of their generalized seizures with other already established models [39]. In the present work, we studied KCC2 as well as β_2-$GABA_AR$ and β_3-$GABA_AR$ subunit expressions in the GASH:Sal strain both at rest and after sound-induced repeated seizures in different brain regions to elucidate their role in the etiology of this epileptic syndrome.

2. Materials and methods

2.1. Animals

The seizure-prone hamster (GASH:Sal) strain employed in these studies was developed at the University of Salamanca. This strain derives from one original epileptic hamster which appeared spontaneously at the University of Valladolid and gave rise to the first seizure-prone hamster strain called GPG:Vall (Gómez-Palomo–Gómez genetically epilepsy-prone hamster, Valladolid). The GPG:Vall strain eventually lost fertility and is now extinct. Before that happened, some individuals were transferred to the University of Salamanca where a new strain was developed (GASH:Sal: genetic audiogenic seizure-prone hamster, Salamanca). For details, see [36,37]. The GASH:Sal strain phenotype is autosomal recessive. Animals' susceptibility to seizures starts around postnatal day 18, which is the time of hearing onset for this species. The strain has the following behavior after sound stimulation: phase 1 — post-stimulus latency period, phase 2 — wild running, phase 3 — tonic–clonic seizures, and phase 4 — stupor. Phases 1–3 last for about 30 s. Stupor (phase 4) may last for 15–20 min. The severity of seizures increases with age, reaching a peak around 2–3 months of age. After 6 months, animals only show phases 1 and 2. The epileptic behavioral syndrome was very similar between GASH:Sal and the extinct GPG:Vall strains. However, GPG:Vall showed morphological alterations in the cochlea and diverse auditory nuclei [40] not present in GASH:Sal [36].

All experimental procedures were approved by the University of Castilla-La Mancha Animal Care Committee and were in accordance with the Declaration of Helsinki and the Guidelines of the Directive 2010/63/EU of the European Parliament and of the Council.

2.2. Experimental groups and sound stimulation procedure

Sixteen adult hamsters (8 GASH:Sal + 8 wild-type Syrian hamsters, 70–80 days old) were used. Experimental groups were as follows: sound-stimulated GASH:Sal epileptic hamsters (SE, n = 4), sound-stimulated control hamsters (SC, n = 4), resting (nonstimulated) GASH:Sal epileptic hamsters (RE, n = 4), and resting (nonstimulated) control hamsters (RC, n = 4). Both SE and SC groups were exposed to white noise (1–37 KHz, 30–80 dB, 10 s) twice a day (leaving a 4–5-hour interval) for five days. The procedure was monitored by the same observer for all the animals tested. This protocol was chosen based on previous evidence from our laboratory to ensure the detection of significant variations in protein levels using Western blot techniques. In a pilot study, only 3 exposures to the abovementioned sound stimulus (once a day for 3 consecutive days) failed to alter cellular prion protein (PrPc) levels in any of the brain regions studied. However, the 10 sound-stimulation protocol employed in this paper was effective in increasing PrPc in the inferior colliculus (unpublished data), probably because of an accumulative effect. Also, the latter protocol proved to have significant effects on neuronal nitric oxide synthase expression in several brain regions without causing a kindling effect [37]. Nevertheless, the protocol employed may elicit axonal growth, since in a previous study we detected an increase in growth-associated protein 43 in the inferior colliculus (unpublished results). The resting (nonstimulated) RC and RE groups served as a basal reference for both the effects of the sound stimulation procedure and the genetic strain differences. After observing 41 GASH:Sal generations, we have determined that under standard housing conditions and in the absence of sound stimulation, GASH:Sal individuals (i.e., RE animals) do not undergo seizures. Nonetheless, animals were checked every day, and no seizures were observed.

2.3. Tissue sampling

Subjects from the sound-stimulated groups (SE and SC) were killed after the 10th stimulation (at the end of the last seizure in the case of the SE group). The RE and RC were sacrificed on the same day without any extra manipulation. Animals were anesthetized (ketamine, 75 mg/kg + xylazine, 10 mg/kg), their brains were quickly removed and dissected on ice, and the following 8 areas were collected: pons + medulla (P + M), cerebellum (CER), inferior colliculus (IC), rest of the mesencephalon (rMES), hypothalamus (HYP), striatum (STR), hippocampus (HIPP), and cerebral cortex (CTX). Brain areas were immediately weighed, frozen on dry ice, and kept in a freezer at −80 °C until assayed.

2.4. Western blot analysis

Brain regions from 16 animals were processed for Western blot analysis using a method which was previously described [37]. Briefly, brain regions were homogenized using a Polytron homogenizer (Kinematica AG, Lucerne, Switzerland) in homogenization buffer containing protease inhibitors. Homogenates were centrifuged at 10,000 rpm for