



Hippocampal ether-à-go-go1 potassium channels blockade: Effects in the startle reflex and prepulse inhibition

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HIGHLIGHTS

- Eag1 K⁺ channels blockade in the dentate gyrus of the hippocampus did not modify apomorphine-disruptive effects in the PPI.
- Dentate gyrus surgery inducing decreased startle response was restored by the Eag1 antibody.
- The role of Eag1 K⁺ channels in the startle response merits further investigation.

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ABSTRACT

Recently, our group described the *ether-à-go-go1* (Eag1) voltage-gated potassium (K⁺) channel (Kv10.1) expression in the dopaminergic cells indicating that these channels are part of the diversified group of ion channels related to dopaminergic neurons function. The increase of dopamine neurotransmission induces a reduction in the prepulse inhibition (PPI) of the acoustic startle reflex in rodents, which is a reliable index of sensorimotor gating deficits. The PPI response has been reported to be abnormally reduced in schizophrenia patients. The role of Eag1 K⁺ channels in the PPI reaction had not been revealed until now, albeit the singular distribution of Eag1 in the dentate gyrus of the hippocampus and the hippocampal regulation of the startle reflex and PPI. The aim of this work was to investigate if Eag1 blockade on hippocampus modifies the PPI-disruptive effects of apomorphine in *Wistar* rats. Bilateral injection of anti-Eag1 single-chain antibody into the dentate gyrus of hippocampus did not modify apomorphine-disruptive effects in the PPI response. However, Eag1 antibody completely restored the startle amplitude decrease revealed after dentate gyrus surgery. These potentially biological important phenomenon merits further investigation regarding the role of Eag1 K⁺ channels, mainly, on startle reflex modulation, since the physiological role of these channels remain obscure.

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1. Introduction

Ether-à-go-go-related gene (ERG) potassium (K⁺) channel is mainly expressed in the human and rodents adult brain [23,24]. ERG K⁺ channels belong to a superfamily of voltage-activated K⁺ channels encoded by three distinct gene subfamilies, including *ether-à-go-go* (*eag*), *ether-à-go-go-like* (*elk*), and *ether-à-go-go-related* (*erg*) genes [8,42]. Among them, the Eag K⁺ channel appears to be the only mainly neuron-specific and localized to synapses

[18]. Eag K⁺ channel is represented by the Eag1 gene (KCNH1, Kv10.1) [26,42] and Eag2 (KCNH5, Kv10.2) [19,23]. Highest Eag1 gene expression is found in the cerebral cortex, hippocampus, cerebellum and hypothalamus of human and rat, which greatly overlaps with Eag1 protein expression [24]. However, the function of Eag1 K⁺ channels in the central nervous system is still unknown [26,27]. It was suggested that the lateral diffusion of Eag1 ion channels acts as a dynamical mechanism to accrue these channels to the synaptic terminals, where they should play a pivotal role for synaptic function and plasticity [11]. The expression of the Eag1 K⁺ channel is high in the nigrostriatal pathway and it is co-localized with the dopaminergic cells [7]. This data suggests that these channels may well participate in the physiology of the dopaminergic system [7].

Sensorimotor gating is the ability to gate out irrelevant stimuli, a normal central inhibitory processes expression, and its ability can be accessed through prepulse inhibition (PPI) in humans [2,29,38] and rodents [3,36,37]. Schizophrenia patients exhibit heritable

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deficits in the PPI response [2], and generally, in rodents, PPI deficit is pharmacologically induced through an increase of dopaminergic neurotransmission [3,36,43]. The dopaminergic (D1/D2) receptor agonist apomorphine induces a reduction in the PPI response of the acoustic startle reflex in rodents, which is a reliable index of the sensorimotor gating deficit [35]. The ability of antipsychotics drugs to restore the PPI disruption may be predictive of its antipsychotic effects [9]. Dopaminergic dysfunction remains the central pathophysiological theory of schizophrenia (for review see [15]), however, the schizophrenia physiopathology might also be related to a brain development and plasticity disorder. In this case, the activity and excitability of multiple brain regions including hippocampus and substantia nigra, may be strongly altered [33,34].

Evidence for the hippocampal involvement in the neuropathology of schizophrenia has been found in humans, from in vivo neuropsychology, structural and functional imaging, and also from *post mortem* histological, morphometric and genetic analyses [14]. Specific evidence for the hippocampal regulation of PPI comes from experimental animal studies [5,39]. Recently, K^+ channels have been proposed to play a role in the mechanisms of neural plasticity which are altered in various psychiatric disorders, especially in hippocampus [16]. The dentate gyrus of the hippocampus acts as the “gate keeper” or the “filter” of the hippocampal function [10]. It has been described that dopamine modulates the activity of new dentate granule cells in the dentate gyrus [25]. Also, dopamine acts as a gate on the direct cortical input to the hippocampus, modulating synaptic plasticity and information flow in a frequency-dependent manner [17]. In this context, Eag1 K^+ channel expression in the dentate gyrus of hippocampal neurons [7] becomes interesting.

Because of the lack of specific pharmacological instruments, Eag1 monoclonal antibody may provide an attractive tool to elucidate the functional role of this channel in mammalian brain [24]. Monoclonal antibodies represent a strategy to generate highly selective inhibitors against cell surface molecules, enabling a specific antigen to be distinguished from its close homologues [12,31]. A specific Eag1 antibody was validated and described as the first monoclonal antibody that selectively inhibits a K^+ current in intact cells [12]. Taking advantage of the specificity of the recombinant anti-Eag1 single-chain antibody, and the singular distribution of Eag1 in the hippocampus (Fig. 1) [7] we investigate the effects of Eag1 antibody microinjection into the dentate gyrus of rats after systemic apomorphine treatment.

2. Materials and methods

2.1. Animals

Male Wistar rats ($n=11$; 250–300 g) were used. The experimental protocol followed the guidelines for the care and use of mammals in Neuroscience and Behavioral Research (ILAR, USA). Rats were housed four per cage, under controlled temperature ($23 \pm 1^\circ\text{C}$) and light/dark cycle (12–12 h; lights on at 0600 hours) with food and water constantly available. Tests were performed in the light phase.

2.2. Drugs

Apomorphine (Sigma–Aldrich, Germany) was dissolved in distilled water with 0.1% ascorbic acid and injected subcutaneously (s.c.; 0.5 mg/kg) at 1 ml/kg. Eag1-specific recombinant anti-Eag1 single-chain antibody (1 $\mu\text{g}/1\ \mu\text{l}$ of water RNase free) was bilaterally injected with 30 gauge needles (16 mm) into the dentate gyrus at a volume of 0.5 μL delivered over 60 s using a microdrive injection pump.

2.3. Sensorimotor evaluation

PPI refers to the reduction in the startle magnitude to a startling acoustic stimulus ‘pulse’ when it is shortly preceded by a weaker ‘prepulse’ stimulus [35]. Startle response and PPI were conducted simultaneously in two identical startle systems (Med Associates, Inc., USA) as described before [30].

2.3.1. Pre-test procedure

Rats were subjected to a PPI pre-test session previously to the surgery. All animals selected to conduct the study presented a positive response to the PPI and an average of startle amplitude of 589 ± 107 (arbitrary units).

2.3.2. Post-surgical test procedure

One week after surgery, animals were re-subjected to the PPI test (post-surgical analyses) before apomorphine treatment.

2.4. Surgery

Rats were anesthetized with ketamine/xylazine (10:7, 100 mg/kg; intraperitoneal) and placed in a stereotaxic apparatus. Stainless steel guide cannulae were bilaterally implanted 3 mm above the injection site within the dentate gyrus (antero-posterior, -4 mm ; lateral, $\pm 2.6\text{ mm}$; dorso-ventral, -2.1 mm) [28]. The guide cannulae were kept unobstructed by dummy stylets.

2.5. Experimental design

After surgery for bilaterally hippocampus cannulae implantation all rats showed a significant decrease of their startle amplitude (compared to pre-test condition). Animals were tested repeatedly (pre-test, post-surgical apomorphine, and apomorphine + Eag1 K^+ channel antibody) with a minimum interval of 4 days, with exception of the post surgical test. PPI disruption was induced by acute apomorphine treatment. Microinjection of the anti-Eag1 K^+ channel antibody bilaterally in the dentate gyrus of hippocampus was conducted 20 min after the apomorphine treatment. Two rats were not included in the behavioral analyses (Fig. 2).

2.6. Statistical analyses

The acoustic startle response to pulse alone and to prepulse + pulse was analyzed by repeated measures analysis of variance (RM-ANOVA) with stimuli (four levels: pulse; 81 or 73 or 69 dB prepulse + pulse) as a within-subjects factor and condition (pre or post-surgical or pharmacological treatment; four levels) as between-subjects factors. For the mean %PPI, RM-ANOVA was conducted with intensity of prepulse (three levels) as a within-subject factor, and condition as a between-subjects factor. In the case of significant main effects the RM-ANOVA was followed by Duncan’s test for post hoc comparisons ($P < 0.05$).

3. Results

3.1. Startle amplitude

We found a significant main effect of stimuli ($F(3,96) = 13.017$; $P = 0.000$) and condition ($F(3,32) = 6.48$; $P = 0.001$), but not a significant interaction between stimuli and condition ($F(9,96) = 1.866$; $P = 0.066$). Rats with bilateral cannulae implantation into the dentate gyrus showed a significant decrease in their startle amplitude to the pulse alone compared with the pre-test condition (Fig. 3). Apomorphine systemic treatment induced an increase in the startle amplitude, but this effect was not statistically significant. After Eag1 antibody bilaterally injected into the dentate gyrus of hippocampus

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