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Research report

Behavioural endophenotypes in mice lacking the auxiliary $GABA_B$ receptor subunit KCTD16

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HIGHLIGHTS

- KCTD16 is one of the auxillary subunits of the $GABA_B$ receptor.
- *Kctd16* knockout mice exhibit increased contextual fear memory.
- *Kctd16* knockout mice exhibit attenuated CS fear memory extinction.
- KCTD16 contributes to the $GABA_B$-regulation of emotional memories.

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ABSTRACT

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain and is implicated in the pathophysiology of a number of neuropsychiatric disorders. The $GABA_B$ receptors are G-protein coupled receptors consisting of principle subunits and auxiliary potassium channel tetramerization domain (KCTD) subunits. The KCTD subunits 8, 12, 12b and 16 are cytosolic proteins that determine the kinetics of the $GABA_B$ receptor response. Previously, we demonstrated that *Kctd12* null mutant mice (*Kctd12*$^{-/-}$) exhibit increased auditory fear learning and that *Kctd12*$^{+/-}$ mice show altered circadian activity, as well as increased intrinsic excitability in hippocampal pyramidal neurons. KCTD16 has been demonstrated to influence neuronal excitability by regulating $GABA_B$ receptor-mediated gating of post-synaptic ion channels. In the present study we investigated for behavioural endophenotypes in *Kctd16*$^{-/-}$ and *Kctd16*$^{+/-}$ mice. Compared with wild-type (WT) littermates, auditory and contextual fear conditioning were normal in both *Kctd16*$^{-/-}$ and *Kctd16*$^{+/-}$ mice. When fear memory was tested on the following day, *Kctd16*$^{-/-}$ mice exhibited less extinction of auditory fear memory relative to WT and *Kctd16*$^{+/-}$ mice, as well as more contextual fear memory relative to WT and, in particular, *Kctd16*$^{+/-}$ mice. Relative to WT, both *Kctd16*$^{+/-}$ and *Kctd16*$^{-/-}$ mice exhibited normal circadian activity. This study adds to the evidence that auxillary KCTD subunits of $GABA_B$ receptors contribute to the regulation of behaviours that could constitute endophenotypes for hyper-reactivity to aversive stimuli in neuropsychiatric disorders.

1. Introduction

The $GABA_B$ receptors are G-protein coupled receptors for γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the mammalian central nervous system (CNS) [1]. They are expressed by almost all neurons and glia in the CNS and are major regulators of synaptic transmission and neuronal excitability, thereby modulating emotion and cognition [2,3]. The $GABA_B$ receptors are hetero-multimers composed of principal and auxiliary subunits. The principal subunits $GABA_{B1a}$, $GABA_{B1b}$ and $GABA_{B2}$ form fully functional $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$ recep-

Abbreviations: BLA, basolateral amygdala; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; KCTD, potassium channel tetramerization domain; Nonidet, non-ionic, non-denaturing detergent; NP, nonyl phenoxy-polyethoxylethanol.

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tors that localize to pre- and postsynaptic sites, respectively [4,5]. These receptors can associate with the cytosolic auxiliary potassium channel tetramerization domain (KCTD) subunits KCTD8, KCTD12, KCTD12b or KCTD16. The KCTD proteins bind constitutively to $GABA_{B2}$ to form receptor subtypes that are structurally and functionally distinct [6]. The KCTDs simultaneously bind to the $GABA_B$ receptor and the G-protein, and thereby influence the kinetics of the receptor response *via* stabilization of the G-protein at the receptor and direct effects on G-protein signaling [7].

Postmortem studies have identified decreased $GABA_B$ receptor density in patients with schizophrenia [8,9] and decreased $GABA_{B1}$ and $GABA_{B2}$ protein expression in patients with schizophrenia, bipolar disorder or major depressive disorder (MDD) [10]. Studies in laboratory animals reported increases in $GABA_B$ receptor density and mRNA expression or function in response to administration of antidepressants [11–14]. In addition, $GABA_B$ receptor antagonists showed anxiolytic and antidepressant-like activity in a number of animal models [15–19]. Accordingly, $GABA_B$ receptors have been proposed as a potential treatment target for these major neuropsychiatric disorders as well as for anxiety disorders and addiction [20–23]. The KCTD proteins are also associated with neuropsychiatric disorders. KCTD12 has been most studied: a genome-wide association study of bipolar disorder I patients identified an association with a single nucleotide polymorphism in a region that includes *KCTD12* [24]; a translational microarray study identified *KCTD12*/*Kctd12* up-regulation in the amygdala of MDD patients and stressed mice relative to their respective control groups [25]. *KCTD16* was identified as a candidate gene for a congenital partial epilepsy syndrome [26]. A *KCTD8* polymorphism was associated with brain size in female adolescents, with its proposed role being KCTD8 modulation of the adverse effects of prenatal exposure to maternal cigarette smoking on cortical development [27].

The availability of mice mutant for the $GABA_B$ receptor principal subunits has allowed for elucidation of their behavioural roles. Thus, genetic ablation of either $GABA_{B1}$ or $GABA_{B2}$, which results in mice lacking functional $GABA_B$ receptors, induced antidepressant-like behavioural effects [15,28], cognitive impairments [29–31] and alterations in the circadian organization of sleep [32]. The contribution of receptor subtypes to physiological and behavioural $GABA_B$ functions was assessed in mice lacking specific $GABA_{B1}$ subunit isoforms [3–5,33,34]. In this respect, the emotional processing and learning and memory of aversive stimuli have been studied using Pavlovian fear conditioning: an initially neutral stimulus, either a discrete event such as a tone (conditioned stimulus, CS) or the general context, is paired with an aversive unconditioned stimulus (US), typically a foot shock [35]. Fear learning, memory and memory extinction learning are then assessed by measuring freezing to the CS or context [35]. In $GABA_{B1a}^{-/-}$ mice *i.e.* mice lacking the $GABA_{B1}$ subunit that localizes to presynaptic sites, tone CS fear learning and memory were normal relative to wild-type (WT), whilst there was an endophenotype of increased generalized fear to a neutral tone CS and a neutral context. In $GABA_{B1b}^{-/-}$ mice *i.e.* mice lacking the $GABA_{B1}$ subunit that localizes to postsynaptic sites, in contrast, fear learning was completely impaired. These critical and distinct roles of specific $GABA_{B1}$ subunit isoforms reflect their major involvement in pre- and postsynaptic modulation of the long-term potentiation induced by thalamo-cortical inputs to the amygdala [36]. In conditioned taste aversion (CTA), another form of aversive learning and memory involving pairing of normally rewarding sweet taste with malaise induced by lithium chloride injection, $GABA_{B1a}^{-/-}$ mice failed to acquire CTA. $GABA_{B1b}^{-/-}$ mice acquired CTA to a similar extent to WT; however, they differed from WT in terms of a robust deficit in extinction learning across repeated exposure to the sweet taste in the absence of malaise [37]. $GABA_{B1}$ subunit-specific mutant mice have also been studied in terms of circadian activity endophenotypes: $GABA_{B1a}^{-/-}$ mice displayed higher inactive phase activity than WT, whereas $GABA_{B1b}^{-/-}$ mice showed active phase hyperactivity relative to WT [38]. Recently, behavioural studies have been conducted with mice lacking the gene for specific auxillary KCTD subunits. We demonstrated that $Kctd12^{-/-}$ mice have an endophenotype of increased fear learning and that $Kctd12^{+/-}$ mice have an endophenotype of hyperactivity during the inactive phase of the circadian cycle, relative to WT. Furthermore, *in vitro* electrophysiological recordings from the hippocampus, a region of high *Kctd12* expression, revealed an increased intrinsic excitability of pyramidal neurons in $Kctd12^{-/-}$ and $Kctd12^{+/-}$ mice relative to WT mice [39].

The auxillary subunit KCTD16 is highly expressed in a number of brain regions including amygdala and hippocampus [40]. It has been demonstrated to regulate the $GABA_B$ receptor-mediated gating of postsynaptic ion channels [6,41], which influences neuronal excitability, oscillatory network activity and cognitive functions [42–45]. Here we report on experiments conducted with $Kctd16^{-/-}$, $Kctd16^{+/-}$ and WT mice aimed at assessing whether KCTD16 regulates behavioural states that could constitute risk factors (endophenotypes) for neuropsychiatric disorders in terms of fear learning and memory and circadian activity.

2. Material and methods

2.1. Animals and housing

Male and female $Kctd16^{+/-}$ mice on a BALB/c background were paired to generate offspring for each genotype *i.e.* WT, $Kctd16^{+/-}$ and $Kctd16^{-/-}$. Mice were weaned at age 3–4 weeks and ear tissue punches were obtained at age 5–7 weeks for genotyping. All animals were caged as littermate pairs-trios in an individually-ventilated cage system (IVC), and were maintained on a reversed 12:12 h light-dark cycle (white light off at 07:00 h). Temperature was set at 20–22 °C and humidity at 50–60%. Food (Complete pellet, Provimi, Kliba Ltd, Kaiseraugst, Switzerland) and water were both available continuously and *ad libitum*. Mice were handled on three days prior to each experiment, and all tests were conducted between 11:00 h and 16:00 h. Each behavioural experiment was performed with naive males aged 8–16 weeks, derived from at least five different litters. There was no effect of genotype on body weight: WT 24.1 ± 1.4 g, $Kctd16^{+/-}$ 23.8 ± 1.0 g, $Kctd16^{-/-}$ 27.1 ± 1.6 g ($p = 0.46$). All procedures were conducted under permits (170/2012, 1897/2015) for animal experimentation issued by the Veterinary Office of Zurich or Basel-Stadt, Switzerland.

2.2. Genotyping

Mice were genotyped by PCR using genomic DNA extracted from ear tissue. To distinguish between the WT and knock-out *Kctd16* alleles, 34 cycles of PCR with primers P1 (5′-TTT GCC CTT GCC TGC AGG T-3′), P2 (5′-ACC GAG AGG ATG CTG AGT C-3′) and P3 (5′- AGC CAA GCT AGC GAA GTT CC-3′) were employed (annealing at 53 °C for 30 s and extension at 72 °C for 1 min), generating fragments of 245 bp for the WT allele and 386 bp for the knock-out allele.

2.3. Western blotting

Brain membranes were prepared as previously described [29]. Briefly, mouse brains were hemi-dissected and homogenized on ice using a Dounce homogenizer in 10 volumes of buffer containing 4 mM Hepes (pH 7.4), 1 mM EDTA, 1 mM EGTA, and 0.32 M sucrose. Homogenates were cleared by centrifugation at 1000*g* for 10 min, and the supernatants again centrifuged at 40000 g for 30 min. The pellets containing the brain membranes were solubilized by rocking the tubes for 3 h at 4 °C in NP-40 buffer (20 mM