ORIGINAL ARTICLE

KCTD12 modulation of GABA(B) receptor function

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Abstract

The molecular composition and functional diversity of native GABA$_B$ receptors (GABA$_B$R) are still poorly understood, thus hindering development of selective GABA$_B$R ligands. Potassium channel tetramerization domain-containing protein (KCTD) 12 is a GABA$_B$R auxiliary subunit and mouse KCTD12 can alter GABA$_B$R function. In this study, we sought to characterize the effects of human KCTD12 on GABA$_B$R kinetics and pharmacology, using an automated electrophysiological assay. Seizure susceptibility and ethanol consumption were also investigated in a KCTD12 knockout mouse model. Human KCTD12 co-expression altered the kinetics of GABA$_B$R-mediated GIRK channels, speeding rates of both activation and desensitization. Analysis of concentration-response curves showed that KCTD12 coexpression did not alter effects of the agonists GABA or baclofen on GABA$_B$R. KCTD12 coexpression enhanced the potentiating effects of the positive allosteric modulator CGP7930, and its effects on GABA$_B$R activation and desensitization. The function of KCTD12 in vivo was examined, using the KCTD12 knockout mouse model. The knockout mice were more resistant to a pentylenetetrazole proconvulsant challenge suggesting reduced seizure susceptibility. In the two bottle preference test, KCTD12 knockout mice demonstrated a reduced consumption at high ethanol concentrations. In summary, human KCTD12 accelerated the kinetics of GABA$_B$R in vitro, in a manner possibly sensitive to allosteric pharmacological modulation. This study also provides novel in vivo evidence that the interaction between KCTD12 and GABA$_B$R is of physiological significance, and may be a mechanism to more selectively modulate GABA$_B$R.

Abbreviations
CGP7930, 2,6-ditert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol; GABAγ, aminobutyric acid; GIRK, G protein-activated inward rectifying potassium channels; KCTD12, potassium channel tetramerization domain- containing proteins12;

Introduction

GABA$_B$ receptors (GABA$_B$R) are heterodimeric G protein–coupled receptors consisting of the GABA$_{B(1a/b)}$ and GABA$_{B(2)}$ subunits. Upon GABA$_B$R activation, G$_{\alpha i/\alpha o}$ and G$_{\beta\gamma}$ proteins are liberated and mediate postsynaptic slow inhibition via G protein-activated inward rectifying potassium channels (GIRK, also known as Kir). The liberated G$_{\beta\gamma}$ proteins also reduce presynaptic transmitter release by inhibiting voltage-gated calcium channels. GABA$_B$Rs are implicated in a range of diseases and are drug targets for multiple therapeutic indications, including muscle spasticity, seizure, and drug addiction (Bettler et al. 2004). Baclofen is used clinically and is a direct GABA$_B$R agonist. However, due to the nonselective activation of GABA$_B$R, its use is often limited by side effects that can include sedation and motor impairment (Hering-Hanit 1999; Lind et al. 2004). The scope for discovery of

selective $GABA_BR$ ligands is limited because of the lack of molecular diversity of $GABA_BR$ subunit isoforms, and the overlapping pharmacological profiles of the two main receptor combinations (Brauner-Osborne and Krogs-gaard-Larsen 1999; Green et al. 2000). Despite this apparent lack of molecular diversity, the functional repertoire of native $GABA_BRs$ is quite broad and varies across different brain regions, suggesting the existence of modulators of $GABA_BR$ function (Bonanno and Raiteri 1993; Cruz et al. 2004; Hayasaki et al. 2012; Hensler et al. 2012).

$GABA_BR$ can interact with other proteins such as the scaffolding proteins Tamalin and MUPP1 (Kitano et al. 2002; Balasubramanian et al. 2007) and these associated proteins are believed to underlie the functional diversity of $GABA_BR$ and provide potential novel mechanisms to modulate $GABA_BR$ (Lujan and Ciruela 2012). Proteomic analysis in rodent brains identified $GABA_BR$ auxiliary subunits belonging to the potassium channel tetramerization domain- containing protein (KCTD) family. The KCTD proteins identified were KCTD8, 12 and its isoform 12b, and 16 (Bartoi et al. 2010; Schwenk et al. 2010). These KCTD proteins are characterized by the presence of a conserved T1 domain of the voltage gated potassium channels (Stogios et al. 2005), followed by H1 and H2 domains in the carboxy-terminal for KCTD8 and 16, while KCTD12 lacks the H2 domain (Seddik et al. 2012). Functional studies, using mouse KCTD proteins showed that all KCTDs increased activation rate of the $GABA_BR$ response, but only KCTD12 increased desensitization of receptor response (Schwenk et al. 2010; Seddik et al. 2012; Ivankova et al. 2013; Turecek et al. 2014). The $GABA_BR$ response desensitization caused by KCTD12 has been attributed to the H1 domain, whereas the H2 domain was shown to inhibit the effect of H1 domain in KCTD8 and 16 (Seddik et al. 2012). Further studies revealed that KCTD12 mediates the desensitization of $GABA_BR$ response by interacting with the liberated $G_{\beta\gamma}$ subunit upon $GABA_BR$ activation (Turecek et al. 2014). In addition to altering the kinetics of $GABA_BR$ responses, mouse KCTD12 has been shown to increase both $GABA_BR$ agonist potency and plasma membrane expression (Bartoi et al. 2010; Schwenk et al. 2010; Ivankova et al. 2013).

A single-nucleotide polymorphism in the gene promoter region of the human *KCTD12* gene was associated with Bipolar I disorder, a $GABA_BR$ implicated disease (Fatemi et al. 2011; Lee et al. 2011; Sand et al. 2012). The potential role of KCTD12 in neuropsychiatric disorders is further supported by altered response to fear conditioning in KCTD12 knockout mice (Cathomas et al. 2015). However, other $GABA_BR$ implicated diseases such as altered brain excitability and alcohol abuse have not been explored.

The effects of KCTD12 on the $GABA_BR$ kinetics and pharmacology would represent a unique opportunity to discover novel and more selective ways to modulate $GABA_BR$. Earlier work characterized function using mouse proteins (Schwenk et al. 2010; Seddik et al. 2012; Ivankova et al. 2013; Turecek et al. 2014). Here, we sought to examine the effects of human KCTD12 on $GABA_BR$ kinetics, pharmacology of $GABA_BR$ ligands, and to explore the influence of KCTD12 on $GABA_BR$ function in two tests of disorders related to seizure and ethanol intake in the KCTD12 knockout mouse model.

Materials and Methods

Electrophysiology recording in oocytes

Stage V or VI oocytes were surgically removed from *Xenopus laevis* and were prepared as described previously (Petrou et al. 1997). Oocytes were kept in ND96 solution and stored at 16°C. Human cDNAs *GABBR1B* (NM_021903), *GABBR2* (NM_005458), *GIRK1* (NM_002239), *GIRK2* (NM_002240), and *KCTD12* (NM_138444.3) were synthesized by Genscript (Piscataway, NJ), and were subcloned into an oocyte high expression vector (Liman et al. 1992). *KCTD12* was codon optimized for oocyte expression. cDNA's were transcribed in vitro (mMessage mMachine, Ambion, Austin, TX), and 40 nl of capped cRNA was injected into each oocyte by the Roboocyte version 1 (Multi Channel Systems, Reutlingen, Germany). A total of 4–19 ng of cRNA was injected into each oocyte. The ratio of the cRNA mixture was 1:1:1:1:15 for GIRK1:GIRK2:GABBR1B:GABBR2:KCTD12. The cRNA ratio was chosen based on the amount of human KCTD12 required to observe a similar level of relative desensitization, a key signature of KCTD12, in a previous study (Schwenk et al. 2010) as shown in Figure S1. After 2–3 days, two electrode voltage clamp recording was performed, using the Roboocyte version 1. Before recording, the oocytes were placed in the bath solution that contained (in $mmol \cdot L^{-1}$) 52 NaCl, 40 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$ and 5 HEPES, pH 7.4, for a minimum of 20 min to allow basal GIRK current rundown to stabilize (Vorobiov et al. 1998). Oocytes were impaled with electrodes that contained 1.5 $mol \cdot L^{-1}$ K-acetate and 0.5 $mol \cdot L^{-1}$ KCl and were held at −50 mV. All drug application times were 60 sec followed by 6 min wash out. Recording frequency was 100 Hz and temperature was maintained between 20 and 22°C.

KCTD knockout mouse model

The KCTD12 knockout mouse model was kindly provided by Professor Bettler and Dr. Gassmann from the University of Basel (Turecek et al. 2014). All studies

involving the mouse model were carried out in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the Florey Institute Animal Ethics Committee. The KCTD12 knockout mouse model is on a C57/Bl6J background. All animals were maintained in a temperature controlled room, with a 12 h light on/off cycle and free access to food and liquid. Experimenter was blinded to the genotypes in the behavioral studies.

Pentylenetetrazole-induced seizure model

P40-45 mice were injected subcutaneously with 100 mg·kg^{-1} of pentylenetetrazole (PTZ). Mice were placed in a clear chamber immediately after injection and the time to tonic hind limb extension was recorded.

Two bottle preference test

Adult male mice (approximately 9 weeks old) were habituated to the holding room environment. The mice were single housed with access to two bottles of water ad libitum. A week later, one bottle of the water was replaced with 5% (v v^{-1}) ethanol, thus mice were given a choice of ethanol or water. The concentration of ethanol was increased in 5% increments every 2 weeks, up to 20% (Moore et al. 2007). The position of the bottles was changed randomly to prevent side preference. Fluid in bottles was replenished weekly. Bottles were weighed to indicate the fluid consumed from each bottle.

Drugs

GABA and baclofen (Sigma, St. Louis, MO) were prepared in bath solution just prior to experimentation. CGP7930 (Tocris, Bristol, U.K.) was dissolved in DMSO at 10 mmol·L^{-1} concentration and stored at −20°C until use. Pentylenetetrazole (Sigma, St. Louis, MO) was dissolved in 0.9% saline just prior experiments. Ethanol (LabServ, Australia) was diluted with water to desired concentration on a weekly basis.

Data analysis

Electrophysiological data were analyzed, using AxoGraph (AxoGraph Scientific, Sydney, Australia). Changes in GABA$_B$R kinetics were quantified with two parameters: the 20–80% rise time and relative desensitization, which was calculated as 100*(1−(end of agonist application response/maximum agonist response)) (Schwenk et al. 2010). Data from electrophysiology and PTZ-induced seizure model are presented as mean ± S.E.M, and statistical analysis was performed on Graph-Pad Prism (GraphPad

Software, La Jolla, CA). Data from two bottle preference tests are presented as mean ± 95% confidence interval (95% CI) and random-effect generalized least-square regression models were performed, using Stata (StatCorp LP, College Station, TX).

Results

Human KCTD12 co-expression altered GABA$_B$R-activated GIRK kinetics

A single concentration of GABA (100 μmol·L^{-1}) was applied for 60 sec onto oocytes expressing GABA$_B$R with or without co-expressed human KCTD12 subunits (Fig. 1A). Compared to GABA$_B$R only expressing oocytes, KCTD12 co-expression significantly reduced the 20–80% rise time by around two-fold ($P < 0.001$) (Fig. 1B). KCTD12 co-expression also significantly increased the relative desensitization to 54.7 ± 1.8%, as compared to 4.54 ± 0.80% in oocytes expressing only GABA$_B$R ($P < 0.001$) (Fig. 1C). Therefore, co-expression of human KCTD12 altered the activation and desensitization kinetics of GABA$_B$R in similar manner as described with mouse KCTD12 (Schwenk et al. 2010; Seddik et al. 2012; Ivankova et al. 2013; Turecek et al. 2014).

Effects of human KCTD12 on GABA$_B$R agonist pharmacology

Based on altered response kinetics, we next tested whether agonist potencies, GABA or baclofen, were changed by human KCTD12 co-expression. Concentration-response curves were constructed by applying 100 μmol·L^{-1} agonist followed by washout and an agonist concentration ranging from 10 nmol·L^{-1} to 100 μmol·L^{-1}. Agonist concentrations could not be applied cumulatively due to KCTD12-induced desensitization. Concentration response to agonists was normalized to the 100 μmol·L^{-1} response obtained in each oocyte and sigmoidal concentration-response curves were fit by nonlinear regression. Concentration response curves for both GABA and baclofen were similar between oocytes with or without co-expressed KCTD12 (Fig. 2). The logEC$_{50}$ for GABA was −6.43 ± 0.1 in oocytes expressing GABA$_B$R alone and −6.46 ± 0.03 with KCTD12 co-expression. The logEC$_{50}$ for baclofen was −6.26 ± 0.02 for GABA$_B$R-expressing oocytes and −6.16 ± 0.04 for KCTD12 co-expression.

Effects of human KCTD12 on CGP7930 pharmacology

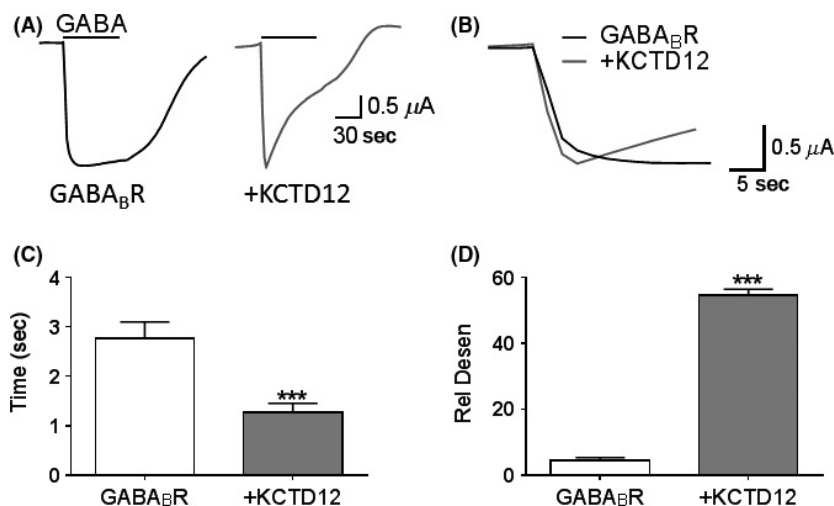KCTD12 is believed to interact with the R2 subunit of GABA$_B$R (Bartoi et al. 2010; Correale et al. 2013). The R2

Figure 1. Effect of human KCTD12 on GABA$_B$R kinetics. (A) Representative current traces from oocytes expressing human GABA$_B$R or co-expressing human KCTD12 (+KCTD12) in response to 1 min application of 100 μmol·L^{-1} GABA. Scale bar applies to both traces. (B) Close up of the rising phase of the GABA-activated response in Fig. 1A. (C) 20–80% rise time of GABA$_B$R ($n = 12$) and +KCTD12 ($n = 10$). (D) Relative desensitization of GABA$_B$R ($n = 12$) and +KCTD12 ($n = 13$). Unpaired *t*-test, ***$P < 0.001$
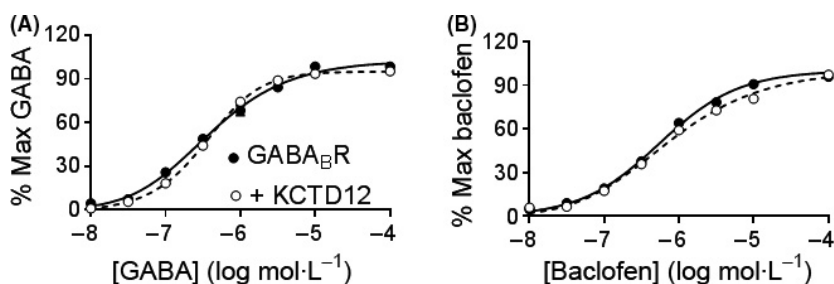

Figure 2. Human KCTD12 did not alter GABA$_B$R sensitivity to agonists. Concentration response curves of (A) GABA and (B) baclofen in the presence and absence of human KCTD12. Responses were normalized to the maximum response in each oocyte. $N = 5$–25 at each agonist concentration.

subunit is thought to contain the binding site of the GABA$_B$R-positive allosteric modulator, CGP7930 (Binet et al. 2004). Therefore, we first examined whether the presence of KCTD12 affects the potentiation of EC$_{20}$GABA by CGP7930. Positive allosteric modulation by CGP7930 (10 μmol·L^{-1}) was determined at the EC$_{20}$ concentration of GABA (100 nmol·L^{-1}) (Fig. 3A). When GABA$_B$R was expressed alone, the average current amplitude before and after CGP7930 were 493 $\pm$ 0.0234 nA and 544 $\pm$ 0.0279 nA, respectively, thus CGP7930 potentiated EC$_{20}$GABA by 10.8 $\pm$ 2.15%. When KCTD12 was co-expressed, average current amplitude before and after CGP7930 were 471 $\pm$ 0.0281 nA and 572 $\pm$ 0.0367 nA, respectively. CGP7930 potentiation was significantly increased to 21.7 $\pm$ 3.55% with KCTD12 co-expression ($P < 0.01$, Fig. 3B).

We next examined whether CGP7930 altered the effects of KCTD12 co-expression on GABA$_B$R kinetics. In

oocytes that only expressed GABA$_B$R, CGP7930 did not affect the 20–80% rise time or relative desensitization in oocytes that only expressed GABA$_B$R was not altered by CGP7930 (Fig. 4). In KCTD12-expressing oocytes, CGP7930 had an effect on receptor kinetics, where it shortened the 20–80% rise time by 18.4% and further accelerated desensitization by 22.5% ($P < 0.01$, Fig. 4).

Reduced seizure susceptibility in KCTD12 knockout mice

Since KCTD12 co-expression profoundly accelerated the desensitization of GABA$_B$R responses in vitro, we hypothesized that deleting KCTD12 expression would increase the amount of inhibition mediated by GABA$_B$R in vivo. Seizure is a result of brain hyper-excitability and a decrease in seizure susceptibility would indicate higher general inhibition in brain. The proconvulsant, PTZ
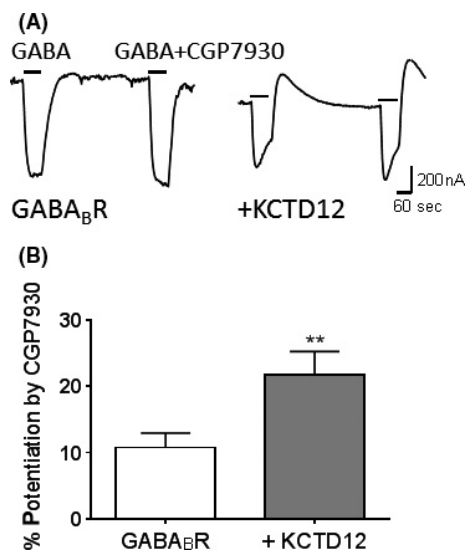
Figure 3. The positive allosteric modulation of CGP7930 is enhanced by KCTD12. (A) Representative current traces from oocytes expressing human GABA$_B$R or co-expressing human KCTD12 (+KCTD12) in response to 1 min application of EC$_{20}$GABA (100 nmol·L^{-1}) followed by washout and then 1 min application of EC$_{20}$GABA and 10 μmol·L^{-1} of CGP7930. Scale bar applied to all traces. (B) The positive allosteric modulator CGP7930 potentiation of EC$_{20}$GABA of oocytes expressing GABA$_B$R ($n = 36$) and +KCTD12 ($n = 28$), unpaired *t*-test, **$P < 0.01$

out of 11 mice showed hind leg extension, the rest of the group did not enter into the seizure state. KCTD12 knockout mice were less susceptible to chemically induced seizure ($P < 0.05$), suggesting that GABA$_B$R mediated inhibition was stronger in the KCTD12 knockout mouse model in comparison with the wild-type mice.

KCTD12 modulates ethanol intake

To further explore the hypothesis that deleting KCTD12 expression would increase the amount of inhibition mediated by GABA$_B$R in vivo, we tested another GABA$_B$R-related behavior, voluntary ethanol intake. Increased GABA$_B$R function decreases ethanol intake and self-administration in animal studies (Colombo et al. 2000, 2006; Besheer et al. 2004; Liang et al. 2006), and several clinical studies have suggested baclofen as a treatment for alcohol dependence (Addolorato et al. 2002, 2007; Morley et al. 2014). In this study, alcohol intake was assayed using two bottle preference test. Random-effect generalized least-square regression models were used due to this method's ability to examine the association between the genotype and the parameters measured (total fluid intake, ethanol intake and preference) at different ethanol concentrations, as well as examining the interaction by genotype and ethanol concentration. Furthermore, appropriate effect size estimates and 95% confidence interval can be reported in addition to P values. During the 8 weeks of experimentation, the average total fluid intake (TFI) of wild type was 127.39 mL·kg^{-1} higher than the KCTD12 knockout mice (95% CI: 6.16–248.62, $P = 0.04$) (Fig. 6A). However, no statistically significant interaction

(100 mg·kg^{-1}) was injected into wild type and KCTD12 knockout mice, and the time to seizure, as indicated by hind leg extension, was measured. For wild type, 6 out of 9 mice showed hind leg extension after 30 min of PTZ treatment (Fig. 5). For KCTD12 knockout mice, only 2
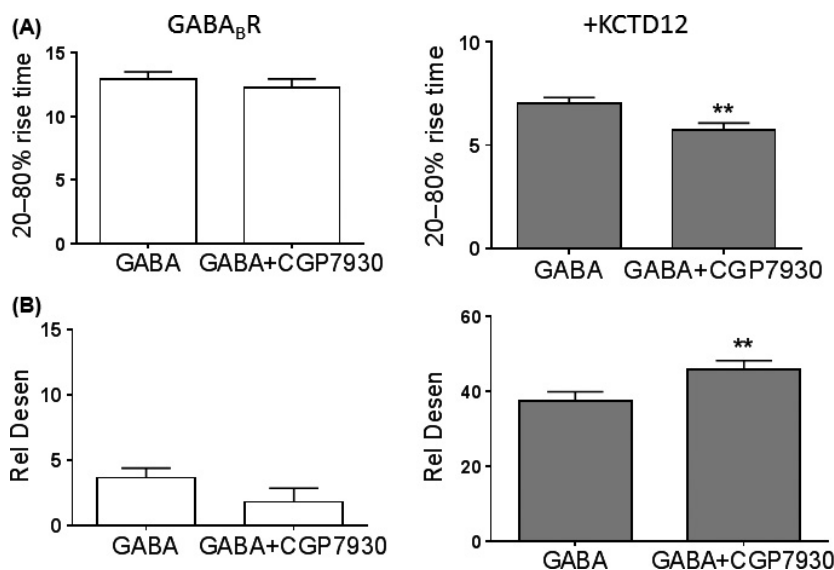

Figure 4. CGP7930 further accentuates the effect of KCTD12 on GABA$_B$R kinetics. Effect of CGP7930 on (A) GABA$_B$R 20–80% rise time and (B) relative desensitization for GABA$_B$R expression only ($n = 24$) or with KCTD12 co-expression ($n = 18$). Paired *t*-test, **$P < 0.01$
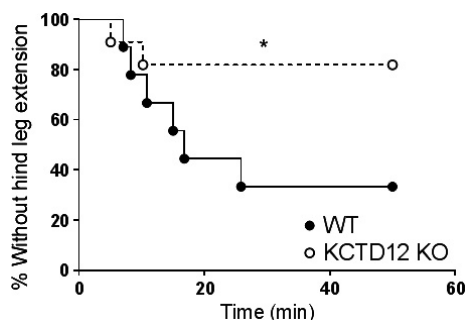
Figure 5. Susceptibility to PTZ-induced seizure in the KCTD12 knockout mouse model. Survival curves of wild type (WT, $n = 9$) and KCTD12 knockout mice (KCTD12 KO, $n = 11$) to subcutaneous PTZ (100 mg·kg^{-1}) induced seizure. Mantel-Cox test, *$P < 0.05$

was found between genotype and ethanol concentration ($P = 0.43$) on TFI. Next the amount of ethanol intake was examined and we observed that during 8 weeks of experimentation, wild type mice consumed on average 21.94 g·kg^{-1} more ethanol than the KCTD12 knockout mice (95% CI: 6.85–37.03, $P = 0.004$) (Fig. 6B). Moreover, an interaction was found between amount of ethanol consumed and ethanol concentration ($P < 0.0001$), indicating that the difference in amount of ethanol consumed between genotypes increased as ethanol concentration increased. Post hoc analysis revealed that the average differences in the amount of ethanol consumed between genotypes were 5.16 g·kg^{-1} (95% CI: −3.66–14.0) at 5% ethanol, 15.57 g·kg^{-1} (95% CI: 1.45–29.7) at 10% ethanol, 21.00 g·kg^{-1} (95% CI: 0.97–40.94) at 15% ethanol and 46.08 g·kg^{-1} (95% CI: 17.11–75.04) at 20% ethanol. To determine whether the wild type simply consumed more fluid than mutants or whether the effect was specific for ethanol, the preference for ethanol, calculated as the percentage of ethanol intake over TFI, was examined. After adjustment for TFI, on average the genotype had no effect on ethanol preference. However, a statistically significant interaction was found between preference and ethanol concentration ($P < 0.0001$) (Fig. 6C). Consistent with the analysis on amount of ethanol consumed, the difference in ethanol preference between genotypes increased as ethanol concentration increased. Post hoc analysis showed that the average differences in ethanol preference between genotypes were −1.06 (95% CI: −15.63–13.5) at 5% ethanol, 6.32 (95% CI: −2.93–15.58) at 10% ethanol, 10.45 (95% CI: 1.15–19.75) at 15% ethanol and 18.02 (95% CI: 8.31–27.73) at 20% ethanol.

Discussion

GABA$_B$R-mediated inhibition of CNS excitability presents a therapeutic opportunity in a variety of diseases, but lack of specific pharmacology has potentially limited its
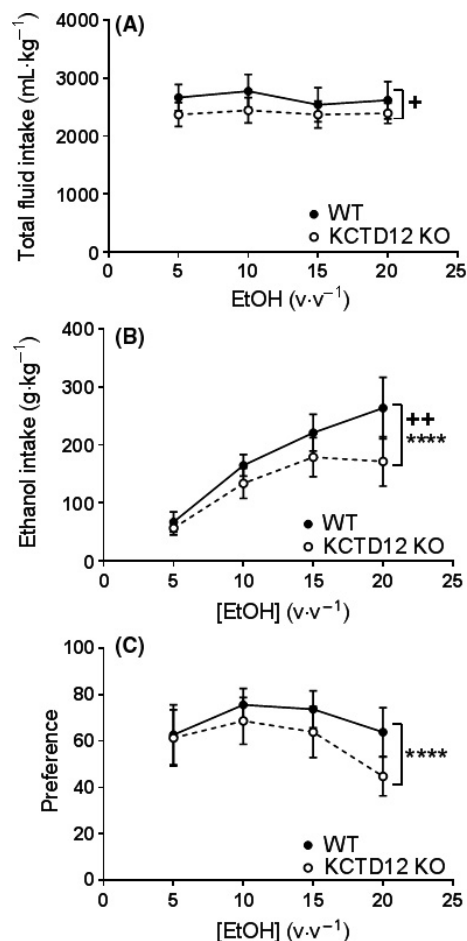

Figure 6. Ethanol preference test in the KCTD12 knockout mouse model. (A) Total fluid intake (water and ethanol containing water) for wild type ($n = 9$) and KCTD12 knockout mice ($n = 10$). Fluid intake was stratified by the concentration of ethanol. (B) The amount of ethanol consumed. (C) Preference for ethanol as indicated by the percentage of ethanol consumed. Note that data is presented as mean ± 95% CI interval. Random-effect generalized least-square test, +$P < 0.05$, ++$P < 0.005$ for significant effect of genotype, *$P < 0.05$ for significant interaction.

application (Bonanno and Raiteri 1993; Cruz et al. 2004; Hayasaki et al. 2012). Recapitulation of native-state conditions in assays may improve the ability to achieve more selective compounds and here we explored the idea that the interaction between KCTD12 and GABA$_B$R may be a more specific way to affect GABA$_B$R for therapeutic benefit. We first developed an electrophysiological assay which showed that the human form of KCTD12 shortened GABA$_B$R responses and enhanced the positive allosteric modulation of CGP7930. Using the KCTD12 knockout mouse model, we showed that deleting KCTD12 expression reduced seizure susceptibility and decreased preference for higher ethanol concentration.

Previous in vitro analysis of KCTD12 used the mouse isoforms (Schwenk et al. 2010; Seddik et al. 2012; Ivankova et al. 2013; Turecek et al. 2014; Rajalu et al. 2015). Here, we report on the ability of human KCTD12 to alter GABA$_B$R kinetics. Similar to the mouse isoform, human KCTD12 accelerated rise time and desensitization of GABA$_B$R. Moreover, the magnitude of modulation by KCTD12 was similar between the mouse and human isoforms (Schwenk et al. 2010). We examined two GABA$_B$R agonists, GABA and baclofen, and found that their potencies were not affected by KCTD12 co-expression. These results are in contrast to a study that showed co-expression of mouse KCTD12 increased baclofen potency on GABA$_B$R-activated calcium channel currents in CHO cells (Schwenk et al. 2010). A recent binding assay study proposed that mouse KCTD12 increases GABA$_B$R agonist potency via effects on G-protein signaling, instead of affinity (Rajalu et al. 2015). Since there are known differences between endogenous mammalian and oocyte G-protein signalling, differences between the baclofen concentration-response curves in the earlier study and in present study may be due to the different heterologous expression systems used. However, in this study, expression of human KCTD12 altered the response of GABA$_B$R's to CGP7930 by enhancing the extent of positive modulation and also by accelerating activation and inactivation kinetics. A recent study using mouse KCTD12 and a different GABA$_B$R positive allosteric modulator GS39783 also observed the accelerated GABA$_B$R kinetics, yet GS39783 positive allosteric modulation was not enhanced by mouse KCTD12 co-expression (Rajalu et al. 2015). The mechanism underlying this discrepancy is unclear but could be due to the potential differences in species, measuring methods and heterologous expression system used.

Proteomic analysis showed that KCTD12 was bound to GABA$_B$R via a constitutive interaction with G$\beta\gamma$ protein (Turecek et al. 2014). Upon GABA$_B$R activation, KCTD12 is believed to mediate desensitization by directly binding liberated G$\beta\gamma$ proteins thereby interfering with G$\beta\gamma$ protein binding and activation of GIRK channels. This activity-dependent binding of KCTD12 to G$\beta\gamma$ protein was termed dynamic binding (Turecek et al. 2014). One interpretation of our CGP7930 result is that CGP7930 binding of GABA$_B$R reduces the amount of constitutively bound KCTD12 and that enhanced currents are due to the consequent greater initial liberation of non-KCTD12 bound G$\beta\gamma$ protein. This is followed by a greater amount of dynamic binding caused by higher levels of free KCTD12. This scenario could explain the greater extent of modulation and the faster activation and deactivation kinetics in the presence of CGP7930 during activation GABA$_B$R in the presence of KCTD12.

Despite our increasing knowledge of the details of KCTD12 signaling, the physiological relevance of KCTD12 remains elusive. The KCTD12 homozygous knockout mice displays increased fear learning to conditioned stimulus (Cathomas et al. 2015). Increased fear learning was also observed in rats treated with baclofen (Heaney et al. 2012), suggesting that KCTD12 deletion has a similar effect to increased GABA$_B$R function. Pharmacological enhancement of GABA$_B$R function in seizures (Mares 2012) and ethanol intake (Colombo et al. 2000, 2006; Addolorato et al. 2002, 2007; Besheer et al. 2004; Liang et al. 2006). Here, we specifically examined the role of KCTD12 in the PTZ-induced seizures and voluntary ethanol consumption. Our studies showed that KCTD12 knockout mice were protected from PTZ-induced seizures and showed reduced preference for high concentrations of ethanol compared to wild types. This is consistent with the idea that a lack of KCTD12 is enhancing GABA$_B$R function in the knockout mouse.

In summary, our study showed that the co-expression of human KCTD12 shortened GABA$_B$R responses. We also found that although the expression of human KCTD12 subunit did not alter the potency of GABA$_B$R agonists, it did increase the allosteric modulation of GABA$_B$R by CGP7930. in vivo studies in the KCTD12 knockout mice confirmed the in vitro notion that deletion of KCTD12 resembles GABA$_B$R enhancement, demonstrating a potential novel target for regional and specific modulation of GABA$_B$R function.

Acknowledgements

Author Contributions

Participated in research design: All authors. Conducted experiments: Li, Reid. Performed data analysis: Li, Walker, Churilov, Lawrence, Reid, Petrou. Wrote or contributed to the writing of the manuscript: All authors.

Disclosure

None declared.

References

Addolorato G, Caputo F, Capristo E, Domenicali M, Bernardi M, Janiri L, et al. (2002). Baclofen efficacy in reducing alcohol

craving and intake: a preliminary double-blind randomized controlled study. Alcohol Alcohol 37: 504–508.

Addolorato G, Leggio L, Ferrulli A, Cardone S, Vonghia L, Mirijello A, et al. (2007). Effectiveness and safety of baclofen for maintenance of alcohol abstinence in alcohol-dependent patients with liver cirrhosis: randomised, double-blind controlled study. Lancet 370: 1915–1922.

Balasubramanian S, Fam SR, Hall RA (2007). GABAB receptor association with the PDZ scaffold Mupp1 alters receptor stability and function. J Biol Chem 282: 4162–4171.

Bartoi T, Rigbolt KT, Du D, Kohr G, Blagoev B, Kornau HC (2010). GABAB receptor constituents revealed by tandem affinity purification from transgenic mice. J Biol Chem 285: 20625–20633.

Besheer J, Lepoutre V, Hodge CW (2004). GABA(B) receptor agonists reduce operant ethanol self-administration and enhance ethanol sedation in C57BL/6J mice. Psychopharmacology 174: 358–366.

Bettler B, Kaupmann K, Mosbacher J, Gassmann M (2004). Molecular structure and physiological functions of GABA(B) receptors. Physiol Rev 84: 835–867.

Binet V, Brajon C, Le Corre L, Acher F, Pin JP, Prezeau L (2004). The heptahelical domain of GABA(B2) is activated directly by CGP7930, a positive allosteric modulator of the GABA(B) receptor. J Biol Chem 279: 29085–29091.

Bonanno G, Raiteri M (1993). gamma-Aminobutyric acid (GABA) autoreceptors in rat cerebral cortex and spinal cord represent pharmacologically distinct subtypes of the GABAB receptor. J Pharmacol Exp Ther 265: 765–770.

Brauner-Osborne H, Krogsgaard-Larsen P (1999). Functional pharmacology of cloned heterodimeric GABAB receptors expressed in mammalian cells. Br J Pharmacol 128: 1370–1374.

Cathomas F, Stegen M, Sigrist H, Schmid L, Seifritz E, Gassmann M, et al. (2015). Altered emotionality and neuronal excitability in mice lacking KCTD12, an auxiliary subunit of GABAB receptors associated with mood disorders. Transl Psychiat 5: e510.

Colombo G, Agabio R, Carai MA, Lobina C, Pani M, Reali R, et al. (2000). Ability of baclofen in reducing alcohol intake and withdrawal severity: I–Preclinical evidence. Alcohol Clin Exp Res 24: 58–66.

Colombo G, Serra S, Vacca G, Carai MA, Gessa GL (2006). Baclofen-induced suppression of alcohol deprivation effect in Sardinian alcohol-preferring (sP) rats exposed to different alcohol concentrations. Eur J Pharmacol 550(1–3): 123–126.

Correale S, Esposito C, Pirone L, Vitagliano L, Di Gaetano S, Pedone E (2013). A biophysical characterization of the folded domains of KCTD12: insights into interaction with the GABAB2 receptor. J Mol Recognit 26: 488–495.

Cruz HG, Ivanova T, Lunn ML, Stoffel M, Slesinger PA, Luscher C (2004). Bi-directional effects of GABA(B) receptor agonists on the mesolimbic dopamine system. Nat Neurosci 7: 153–159.

Fatemi SH, Folsom TD, Thuras PD (2011). Deficits in GABA (B) receptor system in schizophrenia and mood disorders: a postmortem study. Schizophr Res 128(1–3): 37–43.

Green A, Walls S, Wise A, Green RH, Martin AK, Marshall FH (2000). Characterization of [(3)H]-CGP54626A binding to heterodimeric GABA(B) receptors stably expressed in mammalian cells. Br J Pharmacol 131: 1766–1774.

Hayasaki H, Sohma Y, Kanbara K, Otsuki Y (2012). Heterogenous GABA(B) receptor-mediated pathways are involved in the local GABAergic system of the rat trigeminal ganglion: possible involvement of KCTD proteins. Neuroscience 218: 344–358.

Heaney CF, Bolton MM, Murtishaw AS, Sabbagh JJ, Magcalas CM, Kinney JW (2012). Baclofen administration alters fear extinction and GABAergic protein levels. Neurobiol Learn Mem 98: 261–271.

Hensler JG, Advani T, Burke TF, Cheng K, Rice KC, Koek W (2012). GABAB receptor-positive modulators: brain region-dependent effects. J Pharmacol Exp Ther 340: 19–26.

Hering-Hanit R (1999). Baclofen for prevention of migraine. Cephalalgia 19: 589–591.

Ivankova K, Turecek R, Fritzius T, Seddik R, Prezeau L, Comps-Agrar L, et al. (2013). Up-regulation of GABA(B) receptor signaling by constitutive assembly with the K+ channel tetramerization domain-containing protein 12 (KCTD12). J Biol Chem 288: 24848–24856.

Kitano J, Kimura K, Yamazaki Y, Soda T, Shigemoto R, Nakajima Y, et al. (2002). Tamalin, a PDZ domain-containing protein, links a protein complex formation of group 1 metabotropic glutamate receptors and the guanine nucleotide exchange factor cytohesins. J Neurosci 22: 1280–1289.

Lee MT, Chen CH, Lee CS, Chen CC, Chong MY, Ouyang WC, et al. (2011). Genome-wide association study of bipolar I disorder in the Han Chinese population. Mol Psychiatry 16: 548–556.

Liang JH, Chen F, Krstew E, Cowen MS, Carroll FY, Crawford D, et al. (2006). The GABA(B) receptor allosteric modulator CGP7930, like baclofen, reduces operant self-administration of ethanol in alcohol-preferring rats. Neuropharmacology 50: 632–639.

Liman ER, Tytgat J, Hess P (1992). Subunit stoichiometry of a mammalian K+ channel determined by construction of multimeric cDNAs. Neuron 9: 861–871.

Lind G, Meyerson BA, Winter J, Linderoth B (2004). Intrathecal baclofen as adjuvant therapy to enhance the effect

of spinal cord stimulation in neuropathic pain: a pilot study. Eur J Pain 8: 377–383.

Lujan R, Ciruela F (2012). GABAB receptors-associated proteins: potential drug targets in neurological disorders? Curr Drug Targets 13: 129–144.

Mares P (2012). Anticonvulsant action of GABAB receptor positive modulator CGP7930 in immature rats. Epilepsy Res 100(1–2): 49–54.

Moore EM, Serio KM, Goldfarb KJ, Stepanovska S, Linsenbardt DN, Boehm SL 2nd (2007). GABAergic modulation of binge-like ethanol intake in C57BL/6J mice. Pharmacol Biochem Behav 88: 105–113.

Morley KC, Baillie A, Leung S, Addolorato G, Leggio L, Haber PS (2014). Baclofen for the treatment of alcohol dependence and possible role of comorbid anxiety. Alcohol 49: 654–660.

Petrou S, Ugur M, Drummond RM, Singer JJ, Walsh JV Jr (1997). P2X7 purinoceptor expression in Xenopus oocytes is not sufficient to produce a pore-forming P2Z-like phenotype. FEBS Lett 411(2–3): 339–345.

Rajalu M, Fritzius T, Adelfinger L, Jacquier V, Besseyrias V, Gassmann M, et al. (2015). Pharmacological characterization of GABAB receptor subtypes assembled with auxiliary KCTD subunits. Neuropharmacology 88: 145–154.

Sand PG, Langguth B, Itzhacki J, Bauer A, Geis S, Cardenas-Conejo ZE, et al. (2012). Resequencing of the auxiliary GABA (B) receptor subunit gene KCTD12 in chronic tinnitus. Front Syst Neurosci 6: 41.

Schwenk J, Metz M, Zolles G, Turecek R, Fritzius T, Bildl W, et al. (2010). Native GABA(B) receptors are heteromultimers with a family of auxiliary subunits. Nature 465: 231–235.

Seddik R, Jungblut SP, Silander OK, Rajalu M, Fritzius T, Besseyrias V, et al. (2012). Opposite effects of KCTD subunit domains on GABA(B) receptor-mediated desensitization. J Biol Chem 287: 39869–39877.

Stogios PJ, Downs GS, Jauhal JJ, Nandra SK, Prive GG (2005). Sequence and structural analysis of BTB domain proteins. Genome Biol 6: R82.

Turecek R, Schwenk J, Fritzius T, Ivankova K, Zolles G, Adelfinger L, et al. (2014). Auxiliary GABA receptor subunits uncouple G protein betagamma subunits from effector channels to induce desensitization. Neuron 82: 1032–1044.

Vorobiov D, Levin G, Lotan I, Dascal N (1998). Agonist-independent inactivation and agonist-induced desensitization of the G protein-activated K+ channel (GIRK) in Xenopus oocytes. Pflugers Arch 436: 56–68.

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Dose-dependent effect of human KCTD12 on GABA$_B$R response desensitization.