Behavioral/Systems/Cognitive

Disruption of the Ether-à-go-go K^+ Channel Gene BEC1/KCNH3 Enhances Cognitive Function

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The K^+ channel, one of the determinants for neuronal excitability, is genetically heterogeneous, and various K^+ channel genes are expressed in the CNS. The therapeutic potential of K^+ channel blockers for cognitive enhancement has been discussed, but the contribution each K^+ channel gene makes to cognitive function remains obscure. BEC1 (KCNH3) is a member of the K^+ channel superfamily that shows forebrain-preferential distribution. Here, we show the critical involvement of BEC1 in cognitive function. BEC1 knock-out mice performed behavioral tasks related to working memory, reference memory, and attention better than their wild-type littermates. Enhanced performance was also observed in heterozygous mutants. The knock-out mice had neither the seizures nor the motor dysfunction that are often observed in K^+ channel-deficient mice. In contrast to when it is disrupted, overexpression of BEC1 in the forebrain caused the impaired performance of those tasks. It was also found that altering BEC1 expression could change hippocampal neuronal excitability and synaptic plasticity. The results indicate that BEC1 may represent the first K^+ channel that contributes preferentially and bidirectionally to cognitive function.

Introduction

The K^+ channel genes constitute an evolutionarily related multigene superfamily (Yu et al., 2005). The CNS abundantly express many kinds of K^+ channel genes (e.g., Kv1, Kv2, Kv3, Kv4, KCNH, and KCNQ subfamily genes), even voltage-gated K^+ channels. In the CNS, K^+ channels have been implicated in spontaneous seizures and motor dysfunction on the basis of behavioral studies using mutant animals and linkage studies with inherited diseases. There is much evidence of the involvement of Kv1.1, Kv3.1-3, Kv4.2, BK, KCNQ2, and KCNQ3 in spontaneous seizures and motor dysfunction (Ho et al., 1997; Charlier et al., 1998; Singh et al., 1998; Smart et al., 1998; D'Adamo et al., 1999; Lau et al., 2000; Watanabe et al., 2000; Espinosa et al., 2001; Bernard et al., 2004; Sausbier et al., 2004; Peters et al., 2005; Waters et al., 2006). K^+ channel openers show some potential as anticonvulsants [e.g., the anticonvulsant retigabine is an opener of the K^+ channel KCNQ2 (Main et al., 2000)]. Additionally, the K^+ channel blockers linopirdine and apamin have been reported as potential cognitive enhancers (Vickroy, 1993; Fontana et al., 1994; Stackman et al., 2002), and studying the relationship between the K^+ channels and cognitive function may lead to potential therapeutic applications for K^+ channel modulators.

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BEC1 is a member of the ether-à-go-go (KCNH) family in voltage-gated K^+ channel genes that was originally identified using sequence homology (Miyake et al., 1999). BEC1 elicits an outward current both with transient and steady-state components voltage dependently (Miyake et al., 1999; Trudeau et al., 1999). The transient component has fast-inactivating kinetics, and the steady-state component shows a bell-shaped current–voltage relationship, which has also been observed in ERG, a member of KCNH family. Expression of this K^+ channel is reportedly concentrated in the forebrain including the hippocampal and cortical regions (Engeland et al., 1998; Miyake et al., 1999; Saganich et al., 2001), but its physiological roles have not yet been clarified. In this study, BEC1 knock-out (KO) and overexpression (OVER) mice were generated, and their behavioral performances were analyzed to determine whether this K^+ channel plays a critical role in cognitive function. The results demonstrated that BEC1 is negatively involved in cognitive functions with no abnormal behaviors such as spontaneous seizures or motor dysfunction.

Materials and Methods

Generation of BEC1 KO mice. The BEC1 targeting vector was constructed by inserting the promoter and exon 2–7 regions of the mouse BEC1 gene upstream and downstream of the neomycin-resistance cassette of the pPNT plasmid, respectively. J1 cells, an ES cell line derived from 129 mice, were transfected with the vector, cloned in the presence of G418 and ganciclovir, and screened using Southern hybridization. Chimeric mice were mated with C57BL/6J mice to obtain F_1 heterozygous (Hetero) mice. These F_1 Hetero mice were then crossed with C57BL/6J mice. KO and wild-type (WT) male mice (4- to 19-week-old littermates) from the F_2 and F_5 generations were used for experiments. The genotypes of all the offspring were analyzed via PCR using mouse tail DNA and the primersets for

BEC1 (5′-AGTCACTTCGCCAGGCTGTG-3′ and 5′-GGGGCTGCAGGCAGTAGG-3′) and neomycin (5′-TATGGGATCGGCCATTGAAC-3′ and 5′-CCTCAGAAGAACTCGTCAAG-3′).

Generation of BEC1 OVER mice. The BEC1 transgene expression vector was constructed by inserting an α-CaM-kinase II promoter into the pUC18 plasmid containing human BEC1 cDNA with an intron and polyadenylation signal. The promoter region was isolated from mouse C57BL/6 genomic DNA via PCR with primers specific to the GenBank sequence (accession number AJ222796). The transgenic founders were produced by pronuclear injection of the vector into the fertilized eggs of hybrid mice crossing C57BL/6 with DBA2, and then backcrossed with C57BL/6N mice for production of transgenic offspring. Transgenic and WT male mice (5- to 17-week-old littermates) from the F_2 to F_4 generations were used for experiments. The genotypes of all the offspring were analyzed by PCR using mouse tail DNA and the primers 5′-CGAGGCAAGGAACACAGACA-3′ and 5′-GGGGCTGCAGGCAGTAGG-3′. Both the BEC1 transgene and mouse genomic BEC1 DNA are amplified by this set of primers as two fragments of 245 and 338 bp, respectively. The 338 bp fragment contains a 93 bp intron from genomic DNA.

All experiments were performed in compliance with the regulations of the Animal Ethics Committee of Astellas Pharma.

Detection of BEC1 transcripts and proteins. The expression levels of BEC1 mRNA in the brain region were analyzed using real-time quantitative PCR with a PRISM 7700 or 7900HT Sequence Detection System (Applied Biosystems), SYBR Green (Invitrogen), and first-strand cDNA from total RNA digested with DNase. For KO mice, the primers 5′-CGCTTCGACGGGACGCACAG-3′ and 5′-GCAGTAGACCACAGGGAAGAGC-3′ were used. For OVER mice, the primers 5′-AGTCACTTCGCCAGGCTGTG-3′ and 5′-GGGGCTGCAGGCAGTAGG-3′ were designed based on BEC1 cDNA sequences common to mice and humans (transgene). The reference genes BEC2 (KCNH4) and BEC3 (KCNH8) and the transferrin receptor were analyzed with primer sets, 5′-TGCCCTCCAGGCACACTACT-3′ and 5′-GCACCATGTTGTCCCGGA-3′, 5′-GGCCTCAGGAACGGTACCA-3′ and 5′-TGGGTCAGCTTCCTGCTTG-3′, and 5′-TGTTGTAGTAGGAGCCCAGAG-3′ and 5′-CCTGTTCCCACACTGGACTTC-3′, respectively.

Anti-BEC1 antibodies were generated by immunizing rabbits with a peptide corresponding to amino acids 738–751 or a GST-fusion protein with a C-terminal tail (366 aa) made from human BEC1 and purified using an affinity column coupled with each antigen. The purified antibodies were used in volumes of 0.2–1 μg/ml for Western blot analysis. The antibodies detected a diffuse 140 kDa protein with a minor 120 kDa protein in the mouse brain. Deglycosylation with N-glycosidase F caused the disappearance of the 140 kDa protein and an increase in the 120 kDa protein (data not shown). This indicates that, in the brain, the BEC1 protein is mainly an N-linked glycoprotein with a molecular weight of 140 kDa. These results correspond well with the presence of three N-glycosylation consensus motifs in the amino acid sequence of BEC1. Blots were reprobed with anti-neurofilimant H (Millipore).

Y-maze task. The present experiments were performed according to the method of Maurice and Privat (1997). Each arm of the Y-maze was 40 cm long, 13 cm high, 3 cm wide at the bottom, 10 cm wide at the top, and converged at an equal angle. Each mouse was gently placed at the end of one arm and the number of arm entries during an 8 min period was counted. Alternation was defined as entries into all three arms on consecutive occasions. The number of maximum alternations was the total number of arm entries minus two, and the alternation rate (%) was calculated as follows: (actual alternations/maximum alternations) × 100.

Water maze task. For KO mice, a circular pool (148 cm in diameter) and an invisible circular platform (12 cm in diameter) submerged 1 cm beneath the water surface was used. The training protocol consisted of

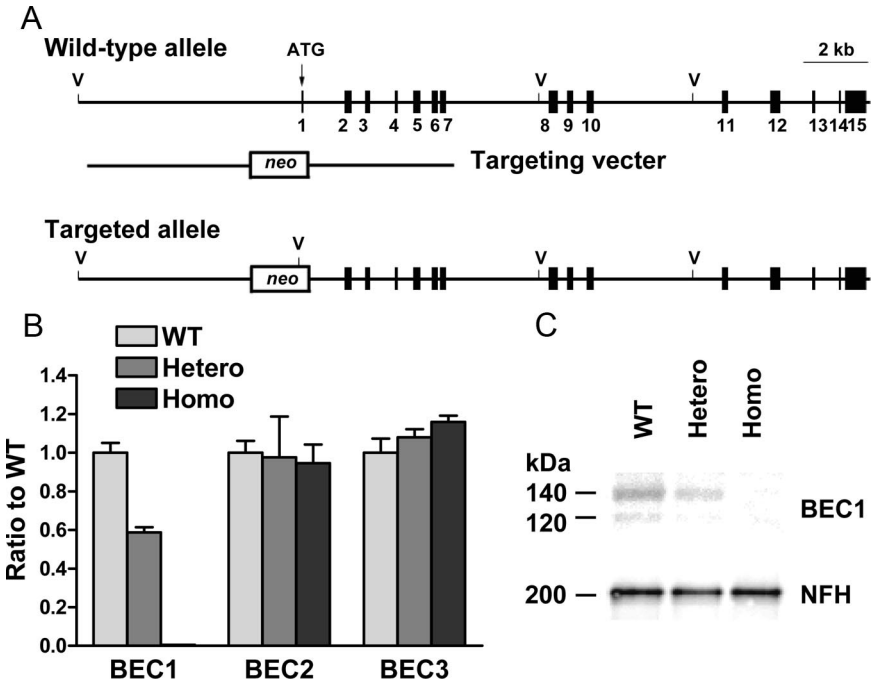

Figure 1. Generation of BEC1 KO mice. ***A***, The construct for targeting the BEC1 gene. The solid boxes indicate exons, whose numbers are shown under the boxes. *neo*, Neomycin-resistance cassette; V, *Eco*RV site. ***B***, Expression of BEC1 mRNA in the forebrain. BEC1, BEC2 (KCNH4), and BEC3 (KCNH8) mRNAs were quantified in three Homo, Hetero, and WT littermates. Changes in expression are indicated as a ratio (mean ± SEM) to WT. Each expression level was normalized using the expression level of the transferrin receptor, a housekeeping gene. ***C***, Expression of BEC1 protein in the forebrain. Each homogenate (10 μg) was immunoblotted with anti-BEC1 antibody and reprobed for neurofilament H (NFH) as a loading control.

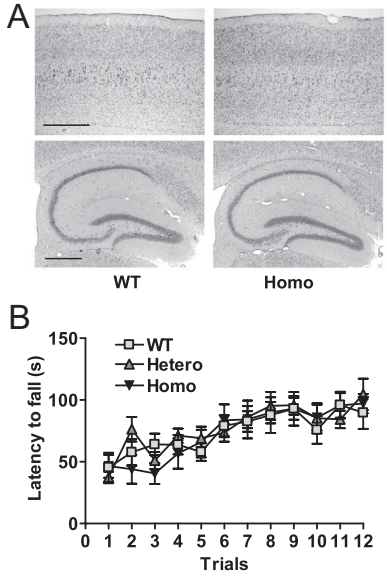

Figure 2. Basic properties of BEC1 KO mice. ***A***, Morphology of KO mice. Left and right, Nissl stains on the cortex (top) and hippocampus (bottom) in the WT and KO littermates, respectively. Scale bar, 0.5 mm. ***B***, Motor coordination on the rotarod. KO and WT mice ($n = 8$) were placed on a 3-cm-diameter rod accelerated from 4 to 40 rpm for 5 min. The latency to fall was measured. Training was performed for 3 consecutive days, receiving four trials per day with a 1 h intertrial interval. Data represent the mean ± SEM.

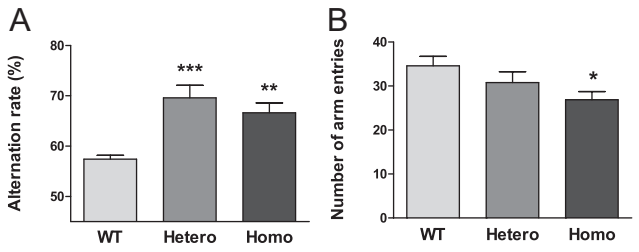

Figure 3. Enhanced spatial working memory of BEC1 KO mice in the Y-maze task. Alternation rate (***A***) and number of arm entries (***B***) were measured for all genotypes ($n = 10$ each). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus WT group, Dunnett's test. Data represent the mean ± SEM.

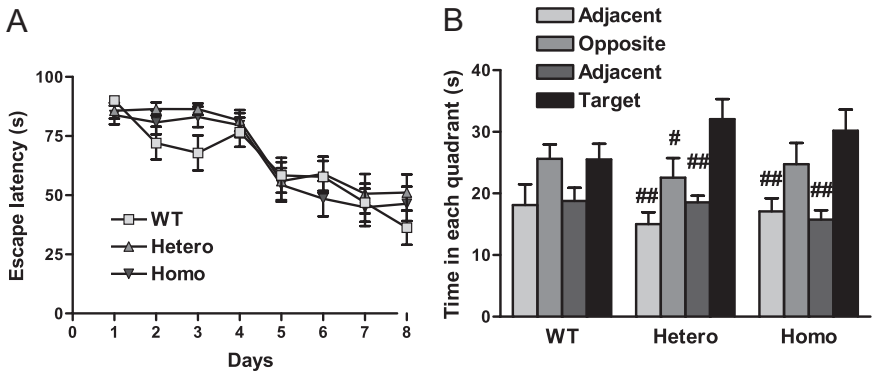

Figure 4. Enhanced spatial reference memory of BEC1 KO mice in the water maze task. Platform escape latency in the training sessions (***A***) and the time spent in each quadrant (***B***) were measured for all genotypes ($n = 20$ each). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus target, Dunnett's test. Data represent the mean ± SEM.

eight sessions with a trial (90 s per trial). The probe test was performed the day after the final session. The platform was removed and the mice were allowed to swim in the pool for 90 s.

For OVER mice, a circular pool (100 cm in diameter) and a circular platform (10 cm in diameter) submerged 0.5 cm beneath the water surface was used. The training protocol consisted of eight sessions with three trials (70 s per trial). The visible platform test was performed during the first three sessions using a randomly located platform with a black triangular pyramid protruding above it. Curtains were drawn around the pool to occlude the extramaze cue. During the next five sessions of the hidden platform test, the extramaze cues were visible. The day after the final session, the platform was removed and the mice were allowed to swim in the pool for 40 s for the probe test. The navigation of the mice was recorded using a video tracking system with SMART software (Panlabs) or Image WMs, a modified version of NIH Image software (O'Hara & Co.). The platform escape latency and the time spent in each quadrant were measured in the training sessions and the probe test, respectively.

Water-finding task. The water-finding task was performed as described previously (Ichihara et al., 1993; Miyamoto et al., 2001). The apparatus consisted of an open field (30 × 50 × 15 cm), whose floor was divided into 15 identical squares by black lines, and whose long wall on the one side had an alcove (10 × 10 × 10 cm). A drinking tube was inserted into the center of the alcove ceiling. The task consisted of two trials: a training trial (the first day) and a test trial (the second day). The mice in each genotype were divided into two groups, trained and untrained. The trained mice of each genotype received both the training and test trials, whereas untrained mice received only the test trial.

In the training trial, each mouse was allowed 3 min to explore the environment, and ambulation was measured by counting the number of lines crossed. The number of times the animal touched a water tube in the alcove (number of approaches) was also counted. It should be noted that water was not delivered from the drinking tube during the training trial. Animals that did not start exploring after 3 min had elapsed or that approached the drinking tube only once or not at all during the 3 min exploratory period were omitted from the test trial.

The trained mice were deprived of water immediately after the training trial. Untrained mice were also deprived of water at the same time. The next day, the mice were again placed individually on the test apparatus for the test trial. The time between entering the alcove and drinking the water (finding latency) were measured up to 360 s. Animals that did not start exploring after 3 min had elapsed were omitted, as in the training trial.

For KO mice and their WT littermates, the tip of the drinking tube was set 5.5 cm above the floor during training and 8.0 cm for the test trials. For WT littermates of OVER mice, the tip of the drinking tube was 7.0 cm above the floor in the test trials. This modification was performed to assess the impairment of cognitive performance because mice could find the water tube more easily. For OVER mice, the tip of the drinking tube was lowered another 0.5 cm in both the training and test trials, because OVER mice were smaller than their WT littermates. There was no significant difference in the number of water tube approaches, or the finding latencies between OVER and WT untrained mice under these conditions (see Results).

Hippocampal slice recording. Mice (4–8 weeks of age) were decapitated under halothane or ether anesthesia. After quickly isolating hippocampi, transverse slices (200–400 μm thick) were cut using a tissue slicer (DTK-1000; Dosaka; or VTS-1200; Leica) in an ice-cold artificial CSF (aCSF) (see below for composition) bubbled with 95% O_2 and 5% CO_2. Slices were then incubated for >1 h in a humidified interface holding chamber (25–31°C). After incubation, a slice was submerged in a recording chamber, held by a platinum grid attached with nylon strings, and superfused with the aCSF solution composed of the following (in mM): 124 NaCl, 3 KCl, 0.5 NaH_2PO_4, 26 $NaHCO_3$, 10 glucose, 2.4 $CaCl_2$, and 1.2 $MgCl_2$, pH 7.3–7.4 equilibrated with 95% O_2 and 5% CO_2.

CA1 pyramidal neurons are visually identified under microscope observation, and whole-cell patch-clamp recordings were made using MultiClamp 700A (Molecular Devices). Recording pipettes were pulled from borosilicate glass capillaries and had a resistance of 3–5 MΩ. The pipette solution contained the following (in mM): 87.5 K-methanesulfonate, 32.5 KCl, 40 HEPES, 0.5 EGTA, 1.0 $MgCl_2$, 12 Na_2 phosphocreatine, 2 ATP-Mg, and 0.5 GTP, pH adjusted to 7.3 with KOH, 290–300 mOsm. Cells with an initial resting membrane potential more positive than −55 mV were excluded from analysis. Action potentials were elicited by current injection through a recording pipette under current-clamp mode. Afterhyperpolarizations were evoked from the resting potential of the cell by applying a 50 ms depolarizing current pulse of sufficient amplitude (300–550 pA) to evoke four overshooting spikes. Accommodation was assessed by counting the number of action potentials during 800 ms depolarizing current pulse.

Field EPSPs (fEPSPs) in the stratum radiatum of the CA1 region were evoked by stimulation of Schaffer collaterals using a bipolar tungsten electrode or a glass pipettes filled with aCSF. The recording electrodes were glass pipettes having a resistance of 3–5 MΩ filled with aCSF. Basal synaptic transmission was monitored at 0.03–0.05 Hz and the signal was amplified with a DAM80 Amplifier (World Precision Instruments) or a Axopatch 1D (Molecular Devices). Stimulus–response curves were recorded with stimulus intensities ranging from 20 to 400 μA. Long-term potentiation (LTP) was induced by high-frequency stimulation (100 Hz for 1 s, a single train) or by a weak theta burst protocol (Murphy et al., 2004), in which two bursts were delivered with an interburst interval of 200 ms. Each burst consisted of four pulses at 100 Hz. Stimulation intensity was adjusted to

give a half-maximal amplitude of the fEPSPs. The records were digitized at 20–50 kHz by an analog-to-digital converter (Digidata 1320A; Molecular Devices) with pClamp9.1 software (Molecular Devices). All experiments were made at room temperature (23–29°C). The statistics were conducted using the number of slices (n) pooled from multiple animals.

Results

Disruption of the BEC1 gene

To determine the physiological function of BEC1 in the CNS, we disrupted the BEC1 gene in mice. The genomic region containing the initiation codon for BEC1 was deleted and replaced with the neomycin-resistant cassette via homologous recombination (Fig. 1*A*). Measurement of mRNA using real-time PCR indicated that homozygous (Homo) mice are almost null, and Hetero mice express approximately one-half of WT transcripts in the forebrain, which is in contrast to closely related genes BEC2 (KCNH4) and BEC3 (KCNH8) (Fig. 1*B*). Western blot analysis using anti-BEC1 antibody also indicated that Homo and Hetero mice are null and one-half, respectively (Fig. 1*C*).

Normal basic properties of KO mice

KO mice showed normal growth and reproductive capability. Body weights of KO mice were similar to their WT littermates (data not shown). No significant developmental abnormalities were observed in any tissues, including the brain. Examination of Nissl-stained brain sections using light microscopy revealed no gross morphological alterations (Fig. 2*A*). The cortex showed a normal layered structure, and the CA1 to CA3 regions and dentate gyrus of the hippocampus, in which BEC1 mRNA is dominantly expressed (Miyake et al., 1999), exhibited no obvious abnormalities. Their general behavior was indistinguishable from that of WT littermates, and neither seizures nor motor dysfunction were observed. In an 80-cm-diameter circular open field, the distance traveled for 3 min of KO mice was not different from that of WT mice: WT, 1085 ± 326 cm; Homo, 1141 ± 134 cm ($n = 6$ each). There were also no performance differences between the KO and WT mice in the rotarod test (Fig. 2*B*). This indicates that spontaneous locomotion and motor coordination are normal in BEC1 KO mice.

Analysis of cognitive performances in BEC1 KO mice

The cognitive performance of BEC1 KO mice were analyzed using the Y-maze, water maze, and water-finding tasks, to assess working memory, reference memory, and attention. The Y-maze and water maze tasks are widely used to test the performance of spatial working and reference memory, respectively (Maurice and Privat, 1997; D'Hooge and De Deyn, 2001). Performance of the water-finding task is dependent on latent learning because motivation to drink the water was not reinforced positively or negatively during the training trials (see Materials and Methods), and selective attention underlies the acquisition of latent learning (Ichihara et al., 1993).

Enhanced spatial working memory of BEC1 KO mice

The Y-maze task was performed to evaluate the working memory of BEC1 KO mice (Fig. 3). BEC1 KO mice exhibited a higher rate of alternation than WT littermates (Fig. 3*A*). Significant differences were observed in both Homo and Hetero mutant mice. Moreover, the number of entries for BEC1 KO mice was fewer than their WT littermates, and there was a significant difference between WT and Homo mice (Fig. 3*B*).

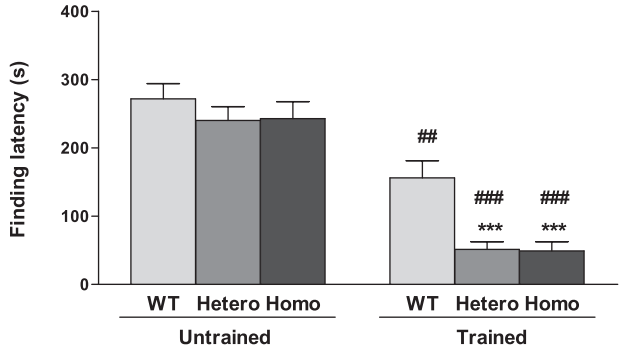

Figure 5. Enhanced latent learning of BEC1 KO mice in the water-finding task. Finding latency was measured in mice trained (WT, $n = 13$; Hetero, $n = 14$; Homo, $n = 13$) or untrained ($n = 14$ for all genotypes) on the previous day. $^{***}p < 0.001$ versus matched WT group, Dunnett's test. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ versus matched untrained group, Student's t test. Data represent the mean ± SEM.

These results suggest that working memory performance is enhanced in BEC1 KO mice.

Enhanced spatial reference memory of BEC1 KO mice

The water maze task with the hidden platform was performed to evaluate the reference memory of BEC1 KO mice (Fig. 4). There was no difference in swim speed between WT and KO mice: WT, 20.4 ± 0.7 cm/s ($n = 20$); Hetero, 21.3 ± 0.5 cm/s ($n = 20$); and Homo, 20.8 ± 0.5 cm/s ($n = 20$) in the first trial. Training protocols with increased difficulty were designed to detect the enhanced performance of KO mice. To increase the difficulty of the task, only one trial per day was performed, and a large pool was used. WT mice did not show enough place preference in the probe test ($p > 0.05$, one-way ANOVA), although the latency to escape to the platform decreased after the training sessions ($p < 0.001$, one-way repeated ANOVA). These results suggest that WT mice may have been in the process of learning the platform location. In the training trials, there was no difference of escape latencies among genotypes ($p > 0.05$, two-way repeated ANOVA). Data of the probe tests were analyzed using two statistical methods: to analyze the time spent in each of the four quadrants within each genotype, and to analyze time spent in the target quadrant across genotypes. In contrast to WT mice, both Homo and Hetero mice exhibited place preference ($p > 0.05$ within WT, $p < 0.01$ within Homo, $p < 0.001$ within Hetero, one-way ANOVA for quadrants), although the differences among genotypes were not significant ($p > 0.05$, one-way ANOVA for genotypes). *Post hoc* analyses (Dunnett's tests) showed that the time Hetero mice spent in the target quadrant was significantly longer than that in any other quadrant, and that Homo mice also spent longer in the target quadrant than either of the adjacent quadrants. These results indicate that both BEC1 KO mice acquired a preference for the target under the same conditions in which WT mice failed. This suggests that the BEC1 KO mice have enhanced special reference memory.

Enhanced latent learning of BEC1 KO mice

The water-finding task was performed to assess the selective attention of BEC1 KO mice (Fig. 5). There was no significant difference in the ambulation or the number of approaches among the WT and BEC1 KO mice during the training trial: WT, 108.1 ± 8.3 and 4.0 ± 0.8 ($n = 13$); Hetero, 107.7 ± 7.9 and 2.9 ± 0.3 ($n = 14$); and Homo, 106.6 ± 4.5 and 3.1 ± 0.4 ($n = 13$), respectively.

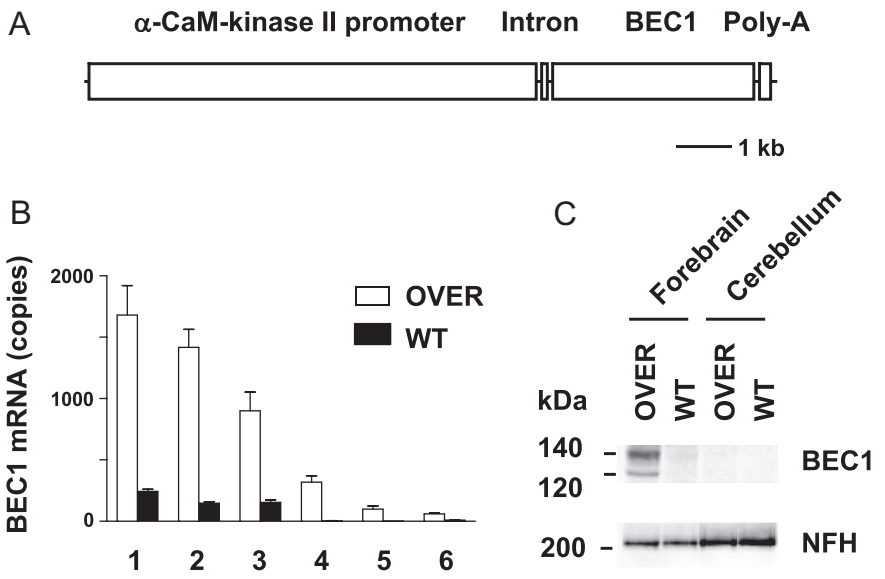

Figure 6. Generation of BEC1 OVER mice. ***A***, The construct for production of OVER mice. ***B***, Overexpression of BEC1 mRNA in OVER mice. The copy number of BEC1 mRNA per nanogram of total RNA (mean ± SEM) was measured in three OVER and WT littermates. Lane 1, Cortex; lane 2, hippocampus; lane 3, striatum; lane 4, thalamus, hypothalamus, and midbrain; lane 5, brainstem; lane 6, cerebellum. ***C***, Overexpression of the BEC1 protein in OVER mice. Each homogenate (10 μg) was immunoblotted with anti-BEC1 antibody and reprobed with anti-NFH.

In the test trial, no significant difference was observed among the three groups of untrained mice (Fig. 5, left columns). Among all genotypes, trained mice had significantly shorter finding latencies than matched untrained mice, and the finding latencies of both Hetero and Homo trained mice were significantly shorter than that of WT trained mice (Fig. 5, right columns). These results suggest that the latent learning associated with selective attention to the drinking tube is enhanced in BEC1 KO mice.

Overexpression of the BEC1 gene

To further investigate the contribution of BEC1 to cognitive function, transgenic mice overexpressing BEC1 in the forebrain, in which it is preferentially expressed, were also produced. The α-CaM-kinase II promoter was used (Fig. 6*A*) because it is available to induce expression of a downstream gene in the forebrain region specifically and postnatally under transgenic conditions (Mayford et al., 1996). Three lines were obtained that showed the expected overexpression selective to the forebrain. One line was used for all the experiments as a representative. Transgene expression was enriched in the cortex, hippocampus, and striatum with low expression in the thalamus, hypothalamus, midbrain, brainstem, and cerebellum (Fig. 6*B*). This expression pattern corresponded well to that of the WT littermates, and the expression levels in the hippocampus were ~10-fold higher. Western blot analysis also indicated overexpression of BEC1 in the forebrain (Fig. 6*C*).

OVER mice grew normally with lower body weights than WT littermates: WT, 33.1 ± 0.7 g, and OVER, 24.8 ± 0.5 g ($n = 24$ each, on conducting the water-finding task). Examination with light microscope showed no gross morphological alterations in brain sections of OVER mice. The OVER mice exhibited no abnormal behaviors such as seizures or motor dysfunctions. Their spontaneous locomotion and motor coordination were also normal (data not shown).

Cognitive impairments of BEC1 OVER mice

The performance of BEC1 OVER mice in the Y-maze task was assessed (Fig. 7*A*). The rate of alternation for OVER mice was less than for WT littermates, although there was no significant difference in the number of arm entries between OVER and WT littermates. These results suggest that working memory performance is impaired in BEC1 OVER mice.

The water maze was performed as a three-phase test composed of the visible platform, hidden platform, and probe tests (Fig. 7*B*). Three trials per day were performed using a conventional pool for training to reduce the difficulty. For the visible platform test, the performance of OVER mice was equivalent to that of the WT mice (Fig. 7*B*, left, days 1–3). This indicates that OVER mice have sufficient visual acuity, swimming ability, and motivation to escape the water. Their swimming speed (17.7 ± 0.7 cm/s; $n = 12$) was also similar to that of the WT mice (18.6 ± 1.2 cm/s; $n = 15$) on day 1. In the hidden platform test, the escape latency of the WT mice decreased after the training sessions ($p < 0.001$, one-way repeated ANOVA) (Fig. 7*B*, left, days 4–8), and a significant preference for the target quadrant was exhibited in the probe test (Fig. 7*B*, right). In contrast, OVER mice showed no alteration of escape latency ($p > 0.05$, one-way repeated ANOVA) or any place preference ($p > 0.05$, one-way ANOVA). The escape latency was significantly longer in OVER mice than in WT littermates ($p < 0.05$, two-way repeated ANOVA). The time spent in the target quadrant was also significantly shorter in OVER mice than that in WT littermates. These results suggest that spatial reference memory is impaired in BEC1 OVER mice.

The water-finding task was also performed to assess the selective attention of BEC1 OVER mice (Fig. 7*C*). There was no significant difference in the ambulation or the number of approaches between WT and OVER mice during the training trial: WT, 80.1 ± 5.3 and 5.3 ± 0.7 ($n = 12$), and OVER, 94.6 ± 12.0 and 4.0 ± 0.4 ($n = 16$), respectively. In the test trial, no significant difference was observed for untrained mice (Fig. 7*C*, left columns). Training had no significant effect on finding latency for OVER mice, but it did significantly shorten that for their WT littermates (Fig. 7*C*, right columns). The change in the finding latency of trained WT mice was significant compared with that of both untrained WT mice and trained OVER mice. These results suggest that the latent learning associated with selective attention to the drinking tube is disturbed in BEC1 OVER mice.

Neuronal excitability and synaptic plasticity of BEC1 mutant mice

To analyze cellular function of BEC1 mutant mice, electrophysiological properties of hippocampal CA1 neurons were investigated. Membrane properties in WT and KO (Homo) neurons, including resting membrane potential, input resistance, spike threshold, half-width, amplitude, and afterhyperpolarization were nearly identical (Table 1). When action potential trains were induced by strong depolarizing-current pulses (typically >600 pA) of 800 ms duration, WT neurons exhibited firing frequency adaptation (Fig. 8*A*). The degree of accommodation was reduced in KO neurons (Fig. 8*B*) ($p < 0.05$, two-way ANOVA). LTP was induced in the Schaffer col-

laterals–CA1 synapses. In OVER mice, tetanus-induced LTP was reduced and the area under the curve (AUC) 35–40 min after tetanus stimuli in OVER mice was significantly lower than that in WT mice (Fig. 8*C*,*D*). Stimulus–response curves and paired-pulse facilitation of OVER mice were almost identical with those of WT mice (data not shown), indicating normal basal transmission of OVER mice. These results suggest that overexpression of BEC1 induces the impairment of synaptic plasticity in the hippocampal CA1 regions. Hippocampal LTP in KO mice slice was induced by tetanus or a weak theta burst stimulus. Obvious difference was found in LTP by neither stimuli between KO and WT mice (Fig. 8*D*).

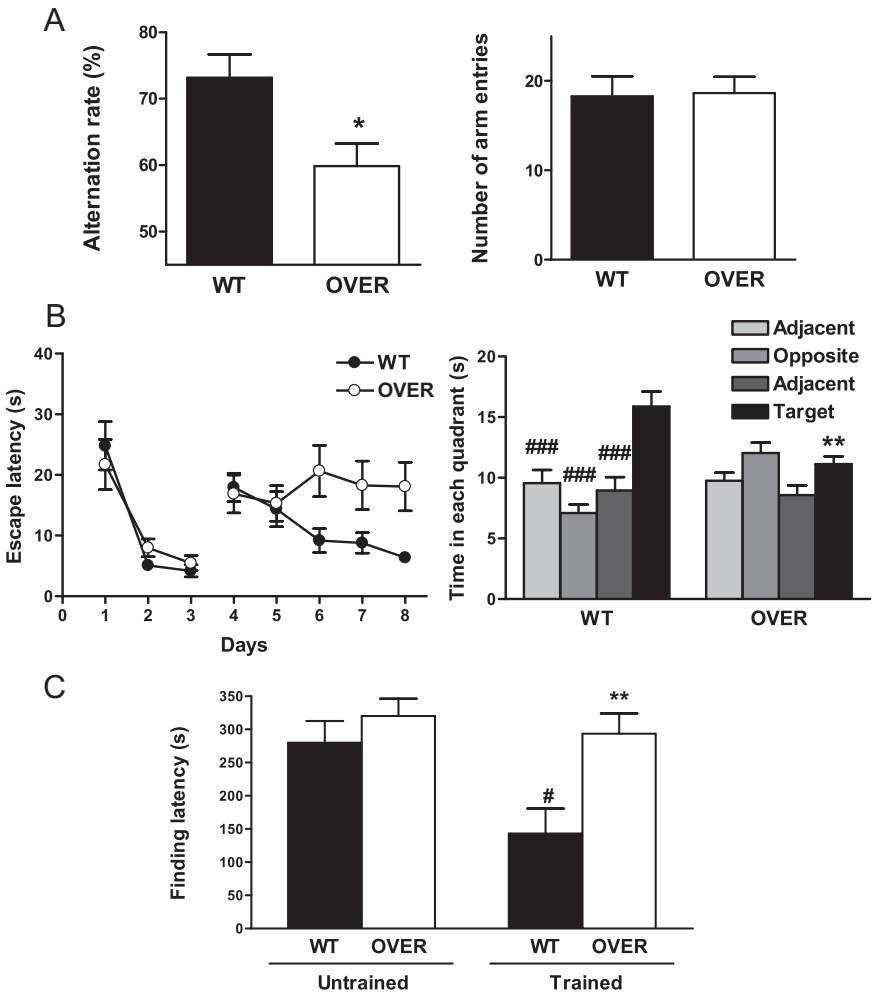

Figure 7. Cognitive impairments of BEC1 OVER mice. ***A***, Spatial working memory in the Y-maze task. Alternation rate and number of arm entries were measured in WT mice ($n = 7$) and OVER mice ($n = 11$). $^{*}p < 0.05$ versus WT group, Student's *t* test. ***B***, Spatial reference memory in the water maze task. Platform escape latency in the training sessions (left) and the time spent in each quadrant (right) were measured in WT ($n = 15$) and OVER ($n = 12$) mice. Visible platform test, Days 1–3; hidden platform test, days 4–8. In the hidden platform test, the escape latency was significantly longer in OVER mice than that in their WT littermates ($p < 0.05$, two-way ANOVA). $^{\#\#\#}p < 0.001$ versus target, Dunnett's test; $^{**}p < 0.01$ versus WT, Student's *t* test. ***C***, Latent learning in the water-finding task. The finding latency was measured in mice trained (WT, $n = 12$; OVER, $n = 8$) or untrained (WT, $n = 12$; OVER, $n = 16$) on the previous day. $^{**}p < 0.01$ versus matched WT group, $^{\#}p < 0.05$ versus matched untrained group, Student's *t* test. Data represent mean ± SEM.

Discussion

Two pieces of evidence that demonstrate the negative contribution of BEC1 to cognitive function are presented in this study: (1) gene disruption enhances cognitive function, and (2) overexpression impairs it. Learning deficits of several K^+ channel mutant mice have been reported: dominant-negative KCNQ2-mutant mice (KCNQ2 is a potential target for linopirdine), SK2-overexpressing mice (SK2 is a potential target for apamin), GIRK4 KO mice, and Kir6.2 KO mice (Wickman et al., 2000; Peters et al., 2005; Choeiri et al., 2006; Hammond et al., 2006). Regarding learning enhancement, there are studies using aged KO mice of Kvβ1.1, an auxiliary subunit of K^+ channels (Need et al., 2003; Murphy et al., 2004). Our study presents evidence that the BEC1 gene plays a role in both the loss- and gain-of-function, which suggests that BEC1 can negatively regulate working memory, reference memory, and attention. A unique property of this KO mouse is that enhanced cognitive performance can be observed in Hetero mice. The enhanced performance of Hetero mice indicates that BEC1 plays a large part in cognitive function.

Mutant mice of K^+ channels often exhibit spontaneous seizures and motor dysfunction (Ho et al., 1997; Charlier et al., 1998; Singh et al., 1998; Smart et al., 1998; D'Adamo et al., 1999; Lau et al., 2000; Watanabe et al., 2000; Espinosa et al., 2001; Bernard et al., 2004; Sausbier et al., 2004; Peters et al., 2005; Waters et al., 2006). These findings are a good reflection of channelopathies, which are inherited diseases caused by mutations of K^+ channel genes in humans. Some examples of channelopathies include benign familial neonatal convulsions (Charlier et al., 1998; Singh et al., 1998), temporal lobe epilepsy (Bernard et al., 2004), neonatal diabetes with epilepsy (Gloyn et al., 2004), generalized epilepsy, and paroxysmal dyskinesia (Du et al., 2005), episodic ataxia (D'Adamo et al., 1999), and adult-onset ataxia (Waters et al., 2006). Because K^+ channels are determinants of neuronal excitability, a K^+ channel deficiency is thought to induce neuronal hyperexcitability resulting in epileptic bursts and/or neuronal degeneration. It is interesting that BEC1 KO mice exhibit altered cognitive performance without seizures or motor dysfunction. Cognitive function selectivity may reflect the preferential expression of BEC1 in the forebrain.

BEC1-KO mice performed better than WT mice in the three different tasks. The Y-maze is a one of the assay systems used to evaluate spontaneous alternation (Maurice and Privat, 1997), and it can evaluate working memory in rodents. The water maze is a task that can evaluate the acquisition and retention of spatial reference memory (D'Hooge and De Deyn, 2001). The water-finding task is thought to be a latent learning paradigm and to be related to the ability to sort sensory information and to attention (Ettenberg et al., 1983; Ichihara et al., 1993; Mamiya et al., 1998). Therefore, a decrease in the activity of BEC1 is considered to enhance the performance of these cognitive domains. The prefrontal cortex is one of the major re-

Table 1. Membrane properties of CA1 pyramidal neurons of WT and KO Homo mice

Genotype	V_m (mV)	R_{in} (MΩ)	Action potential properties			
			Threshold (mV)	Half-width (ms)	Amplitude (mV)	AHP (mV)
WT (10 cells/5 mice)	−68.8 ± 0.6	110 ± 10	−50.1 ± 1.0	1.21 ± 0.04	119 ± 1	2.70 ± 0.34
KO (11 cells/5 mice)	−68.5 ± 1.1	97 ± 7	−48.1 ± 1.2	1.10 ± 0.03	121 ± 1	2.89 ± 0.60

Data are represented as mean ± SEM. V_m, Resting membrane potential; R_{in}, input resistance; AHP, afterhyperpolarization.

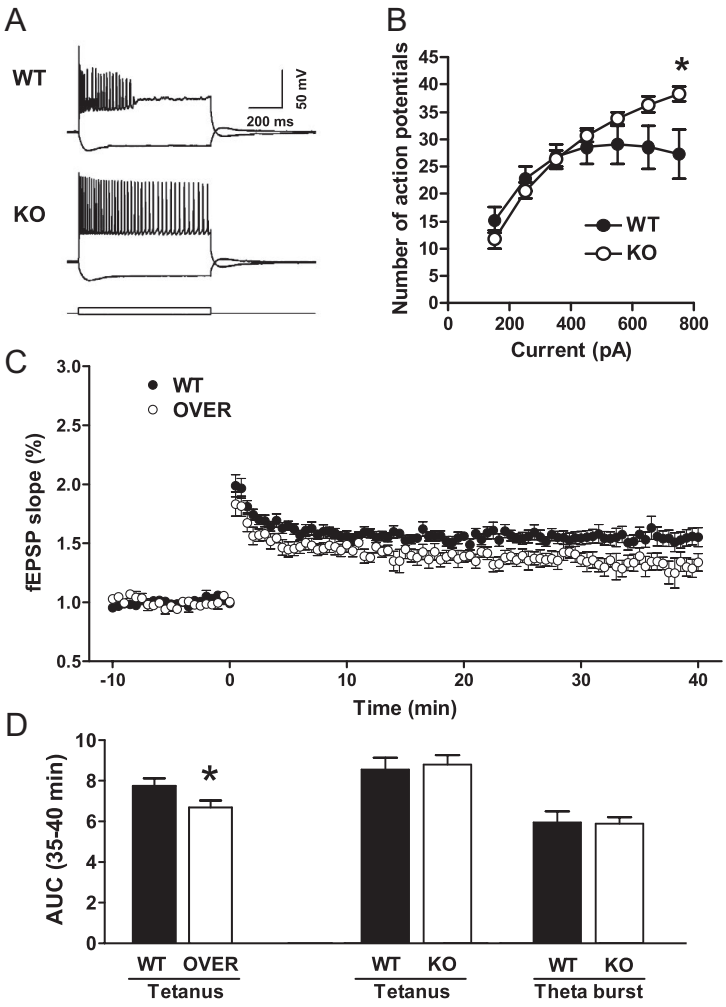

Figure 8. Neuronal function of BEC1. ***A***, Representative recordings from WT (top) and KO mice (Homo; bottom) CA1 pyramidal neurons during tonic current injection (−150 and 750 pA for 800 ms). ***B***, Summary of cell firing from experiments in ***A***. Numbers of action potentials during various intensity of depolarizing current pulse were measured in WT (10 cells/5 mice) and KO mice (10 cells/5 mice). ***C***, Tetanus-induced LTP in WT (12 slices/7 mice) and OVER mice (13 slices/8 mice). fEPSPs evoked by Schaffer collateral stimulation were recorded in the stratum radiatum of the CA1 region. fEPSP slope was normalized with their mean during 10 min before conditioning stimulus, and the conditioning was given at time point of 0. ***D***, AUC of fEPSP slope 35–40 min after stimuli. Data of OVER mice were calculated from data shown in ***C***. Data of KO mice were obtained under the tetanus protocol (WT, 8 slices/6 mice; KO, 10 slices/6 mice) and under the weak theta burst protocol (WT, 6 slices/5 mice; KO, 5 slices/5 mice). *$p < 0.05$ versus WT, Student's t test. Data represent mean ± SEM.

gions that influences working memory performance (Castner and Williams, 2007) and attention (Granon et al., 2000). The hippocampus plays a crucial role in the formation of spatial reference memory (Morris et al., 1982; Poucet et al., 2004). These regions express BEC1 abundantly (Miyake et al., 1999), which suggests that BEC1 may negatively regulate the neural activities involved in cognitive processes in the cortical and hippocampal regions. This explanation is also supported by the observation that transgenic mice overexpressing BEC1 in these forebrain regions exhibited the opposite phenotypes when performing the three behavioral tasks.

To investigate mechanisms underlying altered behaviors of BEC1 mutant mice, hippocampal LTP was analyzed in mutant mice, because it is well characterized as a cellular model of learning and memory. As a result, reduced LTP was detected in OVER mice, whereas obvious alteration has not been detected in KO mice. These results appear to be consistent with data from the water maze task. Learning impairment of OVER mice is apparent, whereas learning enhancement of KO mice is mild. Because many studies using mutant mice suggest good consistency between LTP in the hippocampal CA1 region and reference memory in the water maze (Lee and Silva, 2009), altered LTP in BEC1 mutant mice might be related to learning performance in the water maze task at least. Enhanced LTP was not, however, detected in KO mice. It might be difficult to detect alteration of LTP corresponding to small enhancement of water maze learning in KO mice. Alternatively, remarkable alterations in KO mice were observed in working memory and attention. Other cellular models would be required to explain the involvement in these cognitive domains. We found increased firing frequency of action potentials induced by strong depolarizing stimulation in KO mice. Several K^+ channels (e.g., KCNQ2, Kv1, Kv3, BK, and SK) are involved in firing frequency of neurons (Bond et al., 1999; Rudy et al., 1999; Dodson et al., 2002; Peters et al., 2005; Gu et al., 2007), but the depolarization-dependent regulation of firing frequency is one of the properties of BEC1. Such increased excitability of hippocampal and cortical neuronal network could result in enhancement of these cognitive functions.

Mutant mice are often used to determine the physiological functions of ion channels that conduct well characterized currents in neurons. For example, the behavioral roles of IK_A, IK_V, IK_M, and I_H as they relate to the generation and propagation of action potentials have been analyzed with regard to cognitive function, anxiety, and motor coordination using Kv4.2, Kv3.1, KCNQ2, and HCN1 mutant mice, respectively. However, the BEC1 current does not match any of the endogenous currents that have been characterized in the CNS. Thus, the aim of this study was not to analyze well characterized currents, but to find out the physiological func-

tion of BEC1, a gene identified using sequence homology. Electrophysiological techniques have made it possible to identify and classify many currents on neural soma, dendrites, and axons, but not all currents have been covered. In this paper, we suggest the presence of an unidentified K^+ current that negatively and selectively regulates cognitive domains such as working memory, reference memory, and attention. The purpose of this work is to trigger additional studies on BEC1 as it affects cognitive function. The identification of endogenous BEC1 currents and their mechanisms for modulating cognitive function is yet to be done in detail. Also, the therapeutic potential of BEC1 inhibitors should be studied as it relates to cognitive enhancement.

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