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HCN1 Channels Contribute to the Effects of Amnesia and Hypnosis But Not Immobility of Volatile Anesthetics

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Abstract

Background—HCN1 channels have been identified as targets of ketamine to produce hypnosis. Volatile anesthetics also inhibit HCN1 channels. However, the effects of HCN1 channels on volatile anesthetics *in vivo* is still elusive. This study uses global and conditional HCN1 knockout mice to evaluate how HCN1 channels affect the actions of volatile anesthetics.

Methods—Minimum alveolar concentrations (MAC) of isoflurane and sevoflurane that induced immobility (MAC of immobility) and/or hypnosis (MAC of hypnosis) were determined in wild-type (WT) mice, global HCN1 channel knockout mice (HCN1^{-/-}), floxed HCN1 channel gene (HCN1^{f/f}) mice and forebrain-selective HCN1 channel knockout (HCN1^{f/f}; cre) mice. Immobility of mice was defined as no purposeful reactions to tail-clamping stimulus and hypnosis was defined as loss of righting reflex (LORR). The amnestic effects of isoflurane and sevoflurane were evaluated by fear-potentiated startle in these four strains of mice.

Results—All MAC values were expressed as mean ± SEM. For MAC of immobility of isoflurane, no significant difference was found among wild-type, HCN1^{-/-}, HCN1^{f/f} and HCN1^{f/f}; cre mice (all ~1.24-1.29% isoflurane). For both HCN1^{-/-} and HCN1^{f/f}; cre mice, the MAC of hypnosis for isoflurane (each ~1.05% isoflurane) were significantly increased over their

nonknockout controls: HCN1^{-/-} vs. wild-type (0.86±0.03%, $P<0.001$) and HCN1^{f/f}; cre vs. HCN1^{f/f} mice (0.84±0.03%, $P<0.001$); no significant difference was found between HCN1^{-/-} and HCN1^{f/f}; cre mice. For MAC of immobility of sevoflurane, no significant difference was found among wild-type, HCN1^{-/-}, HCN1^{f/f} and HCN1^{f/f}; cre mice (all ~2.6-2.7% sevoflurane). For both HCN1^{-/-} and HCN1^{f/f}; cre mice, the MAC of hypnosis for sevoflurane (each ~1.90% sevoflurane) was significantly increased over their nonknockout controls: HCN1^{-/-} vs. wild-type (1.58±0.05%, $P<0.001$) and HCN1^{f/f}; cre vs. HCN1^{f/f} mice (1.56±0.05%, $P<0.001$). No significant difference was found between HCN1^{-/-} and HCN1^{f/f}; cre mice. By fear-potentiated startle experiments, amnestic effects of isoflurane and sevoflurane were significantly attenuated in HCN1^{-/-} and HCN1^{f/f}; cre mice (both $P<0.002$ vs. wild-type or HCN1^{f/f} mice). No significant difference was found between HCN1^{-/-} and HCN1^{f/f}; cre mice.

Conclusions—Forebrain HCN1 channels contribute to hypnotic and amnestic effects of volatile anesthetics, but HCN1 channels are not involved in the immobilizing actions of volatile anesthetics.

Introduction

Volatile anesthetics are commonly used in clinical setting. These compounds have been in use for more than 160 years since the application of diethyl ether, and many studies have been performed to investigate the mechanisms by which volatile anesthetics induce general anesthesia. However, the exact mechanisms for different clinical actions of volatile anesthetics are still unclear.¹⁻³

With increasing concentrations of inhaled volatile anesthetics, their actions progress from amnesia (suppression of learning and memory), to hypnosis (unconsciousness) to immobility (no purposeful reactions to nociceptive stimulus).³⁻⁷ Previous studies have demonstrated that different anesthetic endpoints of volatile anesthetics might result from their actions at different neural sites.^{7, 8} For instance, it has been demonstrated that volatile anesthetics produce amnesia and hypnosis at the forebrain region, such as in the cortex and hippocampus⁷, whereas their immobilizing effects might result from effects on spinal cord.⁴⁻¹³ Meanwhile, many molecular targets and neural transmitters such as γ -aminobutyric acid type A (GABA_A), glycine, and glutamate receptors and potassium channels.^{1-3, 14} have been identified as contributing to individual anesthetic endpoint,¹⁻³

HCN (Hyperpolarization-activated cyclic-nucleotide gated) channels are mixed cationic ion channels activated by hyperpolarization.¹⁵ Due to their unique electrophysiological properties, HCN channels play a critical role as pace-makers in the heart and nervous system.^{16, 17} Previous studies have shown that general anesthetics could produce subtype-selective inhibition of HCN channels^{18, 19} and that these channels contribute to general anesthetic actions.^{20, 21} Among HCN channel subtypes (HCN1-4), the HCN1 subtype provides a plausible neuronal mechanism for enhancing cortical synchronization, which is a feature of hypnosis induced by general anesthetics.²² In previous work, forebrain HCN1 channels were found to be a molecular substrate for the hypnotic action of ketamine.^{21, 23} In addition, volatile anesthetics such as halothane, or the more commonly used isoflurane, are

able to inhibit HCN1 channels.^{18, 19} However, whether HCN1 channels contribute to the actions of volatile anesthetics *in vivo* is still unclear.

By using global and forebrain-selective HCN1 knockout mice, we sought to evaluate the roles of HCN1 channels in mediating different end-points of volatile anesthetics *in vivo*. Specifically, the present study investigated the effects of HCN1 channels on the amnestic, hypnotic and immobilizing actions of volatile anesthetics *in vivo*.

Animals

All protocols were approved by the Institutional Animal Experimental Ethics Committee of Sichuan University (Chengdu, Sichuan, China). The experiments were performed on four genotypes of male mice at age of 10-12 weeks, including wild-type C57BL/6J mice, global HCN1 knockout mice (HCN1^{-/-}) mice, HCN1 channel gene with two lox-P sites flanking a region of the fourth exon of HCN1 (HCN1^{f/f}) and forebrain-selective HCN1 knockout mice (HCN1^{f/f}; cre). Wild-type mice were used as nonknockout control of HCN1^{-/-} mice and HCN1^{f/f} mice were used as nonknockout control for HCN1^{f/f}; cre mice. All these mice were housed in standard conditions, with a 12-h light/dark cycle and with free access to food and water. HCN1^{-/-} mice were generated by a standard gene targeting and were bred as previously described.¹⁸ HCN1^{-/-} mice displayed no gross physical or behavioral abnormalities. For HCN1^{f/f}; cre mice, a Cre-loxP strategy²⁴ was used to generate forebrain-selective deletion of HCN1 channels as described previously.²³ Briefly, mice with floxed HCN1 gene (HCN1^{f/f}) were crossed with animals in which Cre-recombinase was expressed selectively in forebrain principal neurons under the control of the CaMKII α -promoter, yielding littermates homozygous for floxed HCN1 alleles that were Cre positive (forebrain-selective HCN1 knockout, known as HCN1^{f/f}; cre mice).

Western blot analysis for HCN1 expression

Cortex, hippocampus, cerebellum and spinal cord tissues from three strains of mice (wild-type, HCN1^{-/-} and HCN1^{f/f}; cre mice) were homogenized and the protein concentrations of the supernatant were determined with a commercial BCA kit (Pierce, Rockford, IL, USA). The supernatant (protein at 20 μ g) was separated by SDS-PAGE and transferred to a nitrocellulose membrane. Primary antibodies of HCN1 (#ab65706, Abcam, Cambridge, MA, USA) and β -actin (#ab1801, Abcam, Cambridge, MA, USA) were used. The membrane was incubated with HRP-conjugated anti-rabbit secondary antibody (1:10,000; Pierce, Rockford, IL, USA) for one hour, and the blot was developed with a Supersignal chemiluminescence detection kit (Pierce, Rockford, IL, USA). A Kodak X-ray Processor 102 (Eastman Kodak, Rochester, NY, USA) was applied for immunoblotting visualization and analyzed by the software Quantity One (Bio-Rad Laboratories, Hercules, CA, USA).

Determination of minimum alveolar concentrations (MAC) in mice

Immobility and hypnotic actions of volatile anesthetics were defined as no purposeful reactions to tail-clamping stimulus and loss of righting reflex (LORR), respectively. Minimum alveolar concentrations (MAC) of isoflurane and sevoflurane that induced immobility (MAC of immobility) and hypnosis (MAC of hypnosis) were determined in wild-type, HCN1^{-/-}, HCN1^{f/f} and HCN1^{f/f}; cre mice, respectively. The mice were put into a

transparent gas-tight plastic chamber (25 × 15 × 12 cm) to inhale isoflurane or sevoflurane, and a heating pad was placed under the chamber to maintain a rectal temperature of between 36 to 38°C. The carrier gas flow (40% O₂/60% N₂) was about 2 L/min. The CO₂ concentration of the chamber was maintained below 5 mmHg by adjusting carrier gas flow. Concentrations of isoflurane and sevoflurane (Abbott pharmacology Ltd. Co., Shanghai, China) were monitored in real time by the RGM monitor (Datex Ohmeda, Louisville, CO, US).

MAC of isoflurane and sevoflurane were determined by up-and-down method as previously described.²⁵ Briefly, MAC of isoflurane and/or sevoflurane was determined on 10 mice for each genotype (n=10). For each trial, 4-6 mice were randomly selected from each genotype and placed in the chamber. Two anesthetic endpoints were determined: hypnosis of mice was defined as loss of righting reflex (LORR) and immobility of mice was evaluated by tail-clamping stimulus (alligator clip, type 85, Newark Electronics, Dublin, CA). For isoflurane, initial concentrations of 0.44% and 0.72% were used, respectively for determinations of hypnosis and immobility. Each concentration was kept for at least 20 min. Concentration of isoflurane was increased by 1.18-fold (e.g. 0.44%, 0.52%, 0.61%, 0.72%, 0.85%, 1% etc.) until successful endpoint (e.g. LORR) was observed. For each mouse, its MAC was the mid-concentration between successful endpoint and previous concentration. For each genotype, MAC was calculated as the average values for each mouse. For sevoflurane, initial concentrations of 1.04% and 1.45%, respectively, were used for determining hypnosis and immobility and each concentration was kept for at least 30 min. Concentration of sevoflurane was also increased by 1.18-fold (e.g. 1.04%, 1.23%, 1.45%, 1.70%, 2% etc.) until successful endpoint (e.g. LORR) was observed. The experimenters were blinded to mice genotypes.

Experiment of fear-potentiated startle

Amnesia induced by isoflurane and/or sevoflurane was evaluated by fear-potentiated startle system (Med Associates, Inc., St. Albans, VT, US). The protocol was modified from previous studies^{26, 27} and were performed over three consecutive days. On the first day, startle responses were evoked by startle noise (105 dB for 50 ms) and baseline startle was determined. Startle responses of mice were determined by an accelerometer and average acceleration of mice was recorded. On the second day, these mice were trained to associate the conditioned noise (70 dB for 5s) with a noxious shock stimulus on their foot (electrical shock, 0.4 mA for 100 ms). The electrical shock was randomly applied 5-10 seconds after conditioned noise and the learning procedure was repeated for 9 times. During the learning procedure, isoflurane and/or sevoflurane were applied at the concentrations of 0.2-fold, 0.3-fold and 0.4-fold MAC of immobility (as above determined). Concentrations of isoflurane and sevoflurane were monitored by the RGM monitor (Datex-Ohmeda, Louisville, CO, US). Air without volatile anesthetics was applied as control. On the third day, all mice were tested for fear-potentiated startle. No electrical shock was applied after the conditioned noise, and startle responses were evoked by startle noise. The sample size was 10 for each genotype.

Statistical Analysis

All the statistical analyses were performed by SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, US). Because of the available mice number and estimated statistical power, sample size was 10/group for behavioral investigations and 4-5/group for western blotting analysis. The MAC value was calculated as the average mid-value of each mouse and expressed as mean $\pm$ SEM. MAC values among different genotypes of mice (wild-type, HCN1^{-/-}, HCN1^{f/f} and HCN1^{f/f}: cre) were compared by one-way ANOVA and multiple comparisons among different genotypes were performed using *post hoc* tests (Bonferroni test for normal distribution and Games-Howell test otherwise). Fear-potentiated startles were calculated as the percentage of increased average acceleration (%) vs. baseline. Fear-potentiated startles of mice were expressed as mean $\pm$ SEM and compared by one-way ANOVA, and multiple comparisons among different genotypes of mice were performed using *post hoc* test (Bonferroni test). Significant threshold was defined as *P* value < 0.05.

Results

Western blot analysis confirmed global and forebrain-selective deletion of HCN1

HCN1 channel subtype protein was detected in all the tissues, including cortex, hippocampus, cerebellum and spinal cord in wild-type mice (Figure 1). As expected for HCN1^{-/-} mice, HCN1 protein (about 100 kDa) was not detected in any of the tissues (Figure 1). For HCN1^{f/f}: cre mice, HCN1 protein was detected in cerebellum and spinal cord but not in cortex and hippocampus, confirming forebrain-selective deletion of HCN1 channel subtype (Figure 1).

MAC of hypnosis was increased in HCN1^{-/-} and HCN1^{f/f}: cre mice whereas MAC of immobility was unaffected

MAC values of both isoflurane and sevoflurane are presented in Table 1. There was no significant difference in MAC of immobility of isoflurane among wild-type, HCN1^{-/-}, HCN1^{f/f} and HCN1^{f/f}: cre mice (*P*=0.972 by ANOVA). For isoflurane (Figure 2), MAC of immobility was 1.25 $\pm$ 0.04%, 1.24 $\pm$ 0.04% (*P*>0.9 vs. wild-type), 1.29 $\pm$ 0.05% (*P*>0.9 vs. wild-type) and 1.26 $\pm$ 0.04% (*P*>0.9 vs. wild-type), respectively for wild-type, HCN1^{-/-}, HCN1^{f/f} and HCN1^{f/f}: cre mice. For sevoflurane (Figure 3), MAC of immobility was 2.62 $\pm$ 0.06%, 2.70 $\pm$ 0.06% (*P*>0.9 vs. wild-type), 2.66 $\pm$ 0.06% (*P*>0.9 vs. wild-type) and 2.63 $\pm$ 0.06% (*P*>0.9 vs. wild-type), respectively for wild-type, HCN1^{-/-}, HCN1^{f/f} and HCN1^{f/f}: cre mice.

MAC of hypnosis was significantly increased in HCN1^{-/-} and HCN1^{f/f}: cre mice compared with controls. For isoflurane (Figure 2), MAC of hypnosis was 0.86 $\pm$ 0.03%, 1.05 $\pm$ 0.03% (*P*<0.001 vs. wild-type), 0.84 $\pm$ 0.03% (*P*>0.9 vs. wild-type) and 1.04 $\pm$ 0.03% (*P*<0.001 vs. HCN1^{f/f}), respectively for wild-type, HCN1^{-/-}, HCN1^{f/f} and HCN1^{f/f}: cre mice. For sevoflurane (Figure 3), MAC of hypnosis was 1.58 $\pm$ 0.05%, 1.89 $\pm$ 0.05% (*P*<0.001 vs. wild-type), 1.56 $\pm$ 0.05% (*P*>0.9 vs. wild-type) and 1.90 $\pm$ 0.05% (*P*<0.001 vs. HCN1^{f/f}), respectively for wild-type, HCN1^{-/-}, HCN1^{f/f} and HCN1^{f/f}: cre mice.

Amnestic actions of isoflurane and sevoflurane were attenuated in both HCN1^{-/-} and HCN1^{f/f}: cre mice

As shown in Figure 4, baseline startle responses were similar among wild-type, HCN1^{-/-}, HCN1^{f/f} and HCN1^{f/f}: cre mice. Fear-potentiated startle was also similar among these mice (no exposure to air flow, isoflurane or sevoflurane).

Fear-potentiated startles in air group were similar among wild-type, HCN1^{-/-}, HCN1^{f/f} and HCN1^{f/f}: cre mice (Figure 5 & Figure 6). Both isoflurane and sevoflurane induced amnesia (suppression of learning and memory) in a concentration-dependent manner.

Fear-potentiated startles were significantly impaired by 0.4 MAC of isoflurane in wild-type, HCN1^{-/-}, HCN1^{f/f} and HCN1^{f/f}: cre mice ($P < 0.001$ vs. air group, Figure 5). However, compared to their nonknockout controls, fear-potentiated startles were significantly increased in both HCN1^{-/-} ($P < 0.001$ vs. wild-type) and HCN1^{f/f}: cre ($P = 0.003$ vs. HCN1^{f/f}) mice. No significant difference was found between HCN1^{-/-} and HCN1^{f/f}: cre mice ($P = 0.197$), indicating that forebrain HCN1 channels contributed to amnesic actions of isoflurane. For sevoflurane at 0.4 MAC of immobility (Figure 6), fear-potentiated startles were also significantly impaired in wild-type, HCN1^{-/-}, HCN1^{f/f} and HCN1^{f/f}: cre mice ($P < 0.001$ vs. air group). However, compared to their nonknockout controls, fear-potentiated startles were significantly increased in both HCN1^{-/-} ($P < 0.001$ vs. wild-type) and HCN1^{f/f}: cre ($P = 0.003$ vs. HCN1^{f/f}) mice. No significant difference was found between HCN1^{-/-} and HCN1^{f/f}: cre mice ($P = 0.740$).

Discussion

In previous studies, HCN1 channels were identified as an underlying molecular target of general anesthetics. Specifically, hypnotic actions of ketamine were significantly attenuated in both HCN1^{-/-} and HCN1^{f/f}: cre mice.^{21, 23} In addition, volatile anesthetics such as isoflurane have been found to inhibit HCN1 channels.¹⁸ These observations represent HCN1 channel as a molecular target for volatile anesthetics, however, the exact role of HCN1 channel in the actions of volatile anesthetics *in vivo* is unclear.

In the present study, we demonstrated that HCN1 channels contributed to amnestic and hypnotic actions of isoflurane and sevoflurane. Because there was no significant difference between HCN1^{-/-} mice and HCN1^{f/f}: cre mice, the neural substrates of these effects likely include forebrain principle neurons. Interestingly, immobility actions of isoflurane and sevoflurane were unaffected by either global HCN1 channel knockout or forebrain-selective HCN1 channel deletion. This observation indicated that HCN1 channels do not contribute substantially to immobilizing actions of volatile anesthetics *in vivo*.

This is not the first time HCN1 channels have been associated with learning and memory.²⁸⁻³⁰ In previous studies, motor learning of mice was impaired by global HCN1 channel knockout²⁸ but spatial learning was improved by forebrain-selective HCN1 channel deletion.²⁹ In addition, intracerebral injection of HCN blocker was found to impair some types of learning and memory.³¹ For amnestic actions of volatile anesthetics in the present study, a fear-potentiated startle paradigm was used because this behavioral assessment was

amenable to administration of inhaled anesthetics. It has been demonstrated that many important brain regions are involved in fear-potentiated startle, such as amygdala and hippocampus.^{32,33}

Previous studies have shown that spinal cord mediates most of the immobility action of volatile anesthetics.⁴ HCN1 channels in spinal cord were deleted in global, but not in forebrain-selective, HCN1 channel knockout mice. If spinal HCN1 channels contribute to immobility actions of isoflurane and sevoflurane, MAC of immobility would be changed in HCN1^{-/-} mice but not in HCN1^{f/f}; cre mice. However, there was no difference in MAC of immobility among wild-type, HCN1^{-/-}, HCN1^{f/f} and HCN1^{f/f}; cre mice, indicating that HCN1 channels might not be involved in the immobility action of volatile anesthetics *in vivo*.

In the present study, isoflurane and sevoflurane could still produce amnestic and hypnotic effects in HCN1^{-/-} and HCN1^{f/f}; cre mice, albeit at significantly higher concentrations. This finding indicates that some other molecular targets might also be involved in the actions of volatile anesthetics. Volatile anesthetics can modulate many other receptors and ion channels, such as γ -aminobutyric acid type A (GABA_A), glycine, and glutamate receptors and potassium channels^{1,2}, and these may also contribute to these amnestic and hypnotic effects. In addition, the different roles of HCN1 channel in amnestic, hypnotic and immobility actions of volatile anesthetics further confirmed the complexity of general anesthetic mechanisms. Therefore, it might be impossible to find an exclusive target for volatile anesthetics and multiple targets might be the most likely scenario.^{3,7}

Some limitations in the present study bear discussion. Firstly, we tested only the effects of isoflurane and sevoflurane, and some other volatile anesthetics may be tested to further demonstrate the generalizability of our conclusions. Secondly, although we found that forebrain HCN1 channels contribute to the amnestic and hypnotic effects of isoflurane and sevoflurane, this is a relatively large brain region, and further refinement of the relevant neural substrate will require mice lines with even more restricted knockout, especially in pyramidal neurons of hippocampus and cortex. Thirdly, although the fear-potentiated startle experiment is a classical method for evaluating the amnestic actions of volatile anesthetics,²⁵⁻²⁷ we did not quantitatively determine activities of mice exposed to volatile anesthetics in amnesia evaluations. Thus, the hypnotic effect of volatile anesthetics might disturb the accuracy of amnesia evaluations. Of note, all the mice involved in amnesic evaluation remained conscious.

In summary, our current study indicates that forebrain HCN1 channels contribute to hypnotic and amnestic actions of volatile anesthetics, whereas spinal HCN1 channels are not involved in the effect of immobility.

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Figure 1.

Analysis of HCN1 channel expression in cortex, hippocampus, cerebellum and spinal cord from wild-type, HCN1^{-/-} and HCN1^{f/f}: cre mice. A: Representative western blots reveal HCN1 protein in all tissues from wild-type mice and no expression in HCN1^{-/-} mice; HCN1 was deleted from cortex and hippocampus in HCN1^{f/f}: cre mice. B: Quantitative analysis of HCN1 channel protein (calculated relative to control). Values were expressed as mean ± SEM (n=4-5/genotype). These results confirmed the global and forebrain-selective HCN1 knockout.

Figure 2.

MAC of isoflurane was shown. Values were expressed as mean $\pm$ SEM. The MAC of immobility was similar among the four strains of mice ($P > 0.9$ by ANOVA). The MAC of LORR was significantly different ($P < 0.001$ by ANOVA) among the four strains of mice. MAC of LORR was significantly increased in HCN1^{-/-} and HCN1^{f/f}: cre mice. *: $P < 0.05$ vs. wild-type mice. #: $P < 0.05$ vs. HCN1^{f/f} mice. HCN1^{f/f}: cre: forebrain-selective HCN1 channels knockout mice; HCN1^{-/-}: global HCN1 knockout mice; HCN1^{f/f}: nonknockout control of HCN1^{f/f}: cre mice; LORR: loss of righting reflex.

Figure 3.

MAC of sevoflurane was shown. Values were expressed as mean $\pm$ SEM. The MAC of immobility was similar among the four strains of mice ($P > 0.9$ by ANOVA). The MAC of LORR was significantly different ($P < 0.001$ by ANOVA) among the four strains of mice. MAC of LORR was significantly increased in HCN1^{-/-} and HCN1^{f/f}: cre mice. *: $P < 0.05$ vs. wild-type mice. #: $P < 0.05$ vs. HCN1^{f/f} mice. HCN1^{f/f}: cre: forebrain-selective HCN1 channels knockout mice; HCN1^{f/f}: nonknockout control of HCN1^{f/f}: cre mice; HCN1^{-/-}: global HCN1 knockout mice; LORR: loss of righting reflex.

Figure 4.

Baseline startle responses of the four strains of mice were determined. A: Baseline startle responses were similar among wild-type, HCN1^{-/-}, HCN1^{f/f} and HCN1^{f/f}: cre mice. The startle responses of mice were evoked by the startle noise. B: Fear-potentiated startle was determined after the conditional association between shock and conditioned noise and were also similar among these strains of mice. Values were expressed as mean $\pm$ SEM. HCN1^{f/f}: cre: forebrain-selective HCN1 channels knockout mice; HCN1^{f/f}: nonknockout control of HCN1^{f/f}: cre mice; HCN1^{-/-}: global HCN1 knockout mice. *: $P > 0.05$.

Figure 5.

Amnestic action of isoflurane determined by fear-potentiated startle. Amnestic action of isoflurane was enhanced in a concentration-dependent manner in all the strains of mice. The amnestic effect of isoflurane was significantly attenuated in both HCN1^{-/-} and HCN1^{f/f}: cre mice at 0.4 MAC. For isoflurane, the MAC used in this experiment was the MAC of immobility which determined in the present study. Potential values were expressed as mean $\pm$ SEM. HCN1^{f/f}: cre: forebrain-selective HCN1 channels knockout mice; HCN1^{f/f}: nonknockout control of HCN1^{f/f}: cre mice; HCN1^{-/-}: global HCN1 knockout mice. *: $P < 0.05$ vs. wild-type mice; #: $P < 0.05$ vs. HCN1^{f/f} mice.

Figure 6.

Amnestic action of sevoflurane determined by fear-potentiated startle. Amnestic action of sevoflurane was enhanced in a concentration-dependent manner in all the strains of mice. The amnestic effect of sevoflurane was attenuated in both HCN1^{-/-} and HCN1^{f/f}: cre mice at 0.2-0.4 MAC. For sevoflurane, the MAC used in this experiment was the MAC of immobility which determined in the present study. Potential values were expressed as mean $\pm$ SEM. HCN1^{f/f}: cre: forebrain-selective HCN1 channels knockout mice; HCN1^{f/f}: nonknockout control of HCN1^{f/f}: cre mice; HCN1^{-/-}: global HCN1 knockout mice. *: $P < 0.05$ vs. wild-type mice; #: $P < 0.05$ vs. HCN1^{f/f} mice.

Table 1

MAC of isoflurane and sevoflurane in the four genotypes of mice

Anesthetic	Isoflurane (%)				Sevoflurane (%)			
	Wild-type	HCN1 ^{-/-}	HCN1 ^{f/f}	HCN1 ^{f/f} ; cre	Wild-type	HCN1 ^{-/-}	HCN1 ^{f/f}	HCN1 ^{f/f} ; cre
MAC of LORR	0.86±0.03	1.05±0.03*	0.84±0.03	1.04±0.03#	1.58±0.05	1.89±0.05*	1.56±0.05	1.90±0.05#
MAC of Immobility	1.25±0.04	1.24±0.04	1.29±0.05	1.26±0.04	2.62±0.06	2.70±0.06	2.66±0.06	2.63±0.06

Data were expressed as mean ± SEM. MAC: minimum alveolar concentration; LORR :Loss of righting reflex; HCN1^{-/-}: global HCN1 knockout mice; HCN1^{f/f}: HCN1 channel gene with two lox-P sites flanking a region of the fourth exon of HCN1 (nonknockout control of HCN1^{f/f}; cre mice); HCN1^{f/f}; cre: forebrain-selective HCN1 knockout mice;

* $P < 0.05$ vs. wild-type;

$P < 0.05$ vs. HCN1^{f/f}.