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Effect of PSD-95/SAP90 and/or PSD-93/Chapsyn-110 Deficiency on the Minimum Alveolar Anesthetic Concentration of Halothane in Mice

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ABSTRACT

Background: The authors have previously shown that the clinically relevant concentrations of inhalational anesthetics dose-dependently inhibit the postsynaptic density protein (PSD)-95, Dlg, and *Z*O-1 domain-mediated protein interactions between *N*-methyl-D-aspartate receptors and PSD-95/synaptic-associated protein (SAP) 90 or PSD-93/Chapsyn-110 and that the knockdown of spinal PSD-95/SAP90 significantly reduces the minimum alveolar concentration (MAC) for isoflurane in rats.

Methods: The authors designed antisense oligodeoxynucleotides based on the mouse PSD-95/SAP90 and PSD-93/Chapsyn-110 messenger RNAs that correspond to their PSD-95, Dlg, and *Z*O-1 domain nucleotides and can specifically knock down the respective proteins. The authors intrathecally injected antisense oligodeoxynucleotides into wild-type and PSD-93/Chapsyn-110 knockout mice to investigate the effect of PSD-95/SAP90 and/or PSD-93/Chapsyn-110 deficiency on halothane anesthesia.

Results: Both PSD-95/SAP90 and PSD-93/Chapsyn-110 antisense oligodeoxynucleotides caused a dose-dependent and significant reduction in the MAC of halothane in wild-type mice. The intrathecal injection of PSD-95/SAP90 antisense oligodeoxynucleotide at different doses (25 and 50 μg) reduced halothane MAC by 40 and 55%, respectively, and intrathecal injection of PSD-93/Chapsyn-110 antisense oligodeoxynucleotide at different doses (12 and 24 μg) reduced halothane MAC by 25 and 53%, respectively. The PSD-95/SAP90 antisense oligodeoxynucleotide showed similar effect on halothane MAC in wild-type and PSD-93/Chapsyn-110 knockout mice, suggesting that the combination of PSD-95/SAP90 knockdown with PSD-93/Chapsyn-110 deletion did not have an additive effect on halothane anesthesia.

Conclusions: The current results indicate that PSD-95/SAP90 and PSD-93/Chapsyn-110 are involved in the molecular mechanisms of halothane anesthesia and that the functional role of PSD-95/SAP90 in halothane anesthesia is not enhanced after PSD-93/Chapsyn-110 deletion.

What We Already Know about This Topic

- Halothane produces anesthesia in mice by a mechanism that involves proteins (postsynaptic density protein [PSD]-95/SAP90 and PSD-93/Chapsyn-110) that interface with the *N*-methyl-D-aspartate receptor in the spinal cord, but the nature of interaction between these proteins is unknown

What This Article Tells Us That Is New

- In mice, intrathecal injection of antisense to PSD-95/SAP90 reduced its expression and reduced minimum alveolar concentration for halothane
- This reduction was similar in mice lacking PSD-93/Chapsyn-110

N-METHYL-D-aspartate (NMDA) receptor activation has been shown to play an important role in the processing of spinal nociceptive information[1–4] and in the determination of the minimum alveolar concentration (MAC) of inhalational anesthetics.[5–11] The NMDA receptor NR2B subunit interacts with the membrane-associated guanylate-like kinase (MAGUK) family of proteins in the postsynaptic density protein (PSD) of the central nervous system through the binding of the *C*-terminal terminus serine-X-valine (X = unspecified amino acid) intracellular motif of the NR2B sub-

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unit to the *N*-terminal PSD-95, Dlg, and ZO-1 (PDZ) domains (a term derived from the names of the first three proteins identified to contain the domain: PDZ) of the MAGUK proteins. The MAGUK family includes four members: PSD-95/synaptic-associated protein (SAP) 90, PSD-93/Chapsyn-110, SAP97, and SAP102. PSD-95/SAP90 and PSD-93/Chapsyn-110 have been identified to attach NMDA receptors to the internal signaling molecules at neuronal synapses.[12,13] This interaction suggests that PSD-95/SAP90 and PSD-93/Chapsyn-110 might be involved in many physiologic and pathophysiologic functions triggered *via* the activation of NMDA receptors in the central nervous system.

In our previous studies, we have performed standard nociceptive behavioral tests after intrathecal injection of PSD-95/SAP90 or PSD-93/Chapsyn-110 antisense oligodeoxynucleotide. We used von Frey filaments to measure mechanical allodynia and also used the method of Hargreaves *et al.*[14] to measure thermal hyperalgesia. Our results have shown that the knockdown of PSD-95/SAP90 or PSD-93/Chapsyn-110 in the spinal cord inhibited inflammatory pain and neuropathic pain, but it had no effect on baseline nociceptive behaviors.[15–17] Furthermore, we have shown that the clinically relevant concentrations of inhalational anesthetics dose-dependently and specifically inhibit the PDZ domain-mediated protein interactions between NMDA receptors and PSD-95/SAP90 or PSD-93/Chapsyn-110.[18] These inhibitory effects are immediate, potent, and reversible and occur at a hydrophobic peptide-binding groove on the surface of the second PDZ domain of PSD-95/SAP90 or PSD-93/Chapsyn-110 in a manner relevant to anesthetic action.[18] These findings reveal the PDZ domain as a molecular target for inhalational anesthetics. We have also found that PSD-95/SAP90 is involved in the central mechanisms of isoflurane anesthesia.[19] However, it is unknown currently whether PSD-95/SAP90 and PSD-93/Chapsyn-110 have functionally similar or distinct roles in inhalational anesthesia. In the current study, we used a combination of genetic knockout and antisense oligodeoxynucleotide knockdown strategies to investigate the effect of deficiency of PSD-95/SAP90 and/or PSD-93/Chapsyn-110 on the threshold for halothane anesthesia and to determine whether the two MAGUK proteins have additive effects on halothane MAC.

Materials and Methods

Animal Preparation

Male C57BL/6J wild-type (WT) and PSD-93/Chapsyn-110 knockout mice weighing 25–30 g were housed up to four per cage on a standard 12-h light–dark cycle with water and food pellets available *ad libitum*. The animals were habituated in a Plexiglas chamber for 60 min before testing. All behavioral testing was carried out between 10:00 AM and 4:00 PM. Animal experiments were carried out with the approval of the Animal Care and Use Committee at Johns Hopkins University and were consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used. The animal assignment (6–8 mice per group) was blinded to the observer for all *in vivo* behavioral testing, including MAC measurement and locomotor function tests.

Intrathecal Injection in Unanesthetized Mice

The intrathecal injections were performed as described previously.[20] In brief, the mouse was held firmly by the pelvic girdle in one hand, while a 10-μl luer tip syringe with a 30-gauge, 0.5-inch needle was held in the other hand at an angle of approximately 20° above the vertebral column. The needle was inserted into the tissue to one side of the L5 or L6 spinous process, so that it slipped into the groove between the spinous and transverse processes. The needle was then moved carefully forward to the intervertebral space as the angle of the syringe was decreased to approximately 10°. A tail flick indicated that the tip of the needle was inserted into the subarachnoid space. The vehicle or drug solution was injected intrathecally in a volume of 5 μl.

Intrathecal Administration of Oligodeoxynucleotides

Antisense oligodeoxynucleotides for PSD-95/SAP90 and PSD-93/Chapsyn-110 were designed based on the messenger RNA sequences of the respective mouse PSD-95/SAP90 and PSD-93/Chapsyn-110 PDZ domains. The controls for the effect of antisense oligodeoxynucleotide included saline and missense oligodeoxynucleotide. The missense oligodeoxynucleotides contained adenosine, thymidine, guanosine, and cytidine nucleotides that were in proportions identical to that of the antisense oligodeoxynucleotide but in randomly assigned sequence. The sequences for the oligodeoxynucleotides were as follows[15,17]: PSD-95/SAP90 antisense oligodeoxynucleotide, 5′-TGTGATCTCCTCATACTC-3′ and missense oligodeoxynucleotide, 5′-AAGCCCTTGTTCCCATTT-3′; and PSD-93/Chapsyn-110 antisense oligodeoxynucleotide, 5′-CCCTCTCCAATGTAATTTC-3′ and missense oligodeoxynucleotide, 5′-ATACTTCCTTTGTCCACCA-3′. The oligodeoxynucleotides were searched to exclude nonspecificity of the antisense oligodeoxynucleotides and to show that the missense oligodeoxynucleotides did not match any confounding sequences in the GenBank database. The oligodeoxynucleotides were made and purified by reversed-phase high-performance liquid chromatography (Integrated DNA Technologies Inc., Coralville, IA) and were reconstituted in saline before administration. Every 24 h for 4 days, the mice were injected intrathecally with saline (5 μl), PSD-95/SAP90 antisense oligodeoxynucleotide (12.5 μg, 25 μg, or 50 μg/5 μl) or its missense oligodeoxynucleotide (50 μg/5 μl), or PSD-93/Chapsyn-110 antisense oligodeoxynucleotide (6 μg, 12 μg, or 24 μg/5 μl) or its missense oligodeoxynucleotide (24 μg/5 μl).

Measurement of Halothane MAC

The measurement of halothane MAC value was carried out as described previously with minor modification.[21–23] On the day after the last intrathecal injection, mice were placed in individual Plexiglas chambers, and a rectal temperature probe was inserted

under light general anesthesia (~0.6% halothane). Each chamber was fitted with a rubber stopper at one end through which the tail of mouse and the rectal temperature probe protruded. Groups of four mice were given halothane in oxygen (4 l/min total gas flow). A gas sample was continuously drawn, and the anesthetic concentration was measured with an agent analyzer (Ohmeda 5250 RGM, Louisville, CO). The temperature of the mice was kept at 36°–38°C with heat lamps throughout the experiment. Mice initially breathed approximately 1.5% halothane for 60 min. Then, a 15-cm hemostatic forceps was applied to the tail for 1 min, and the mice were observed for a movement in response to the stimulation. Motor activity (gross movements of the head, extremities, and/or body) was considered as a positive response. Next, the anesthetic concentration was increased (or decreased) by 0.1%. After 10 min of equilibration, the tail was stimulated again. Only the middle third of the tail was used for tail clamping, and the clamp was always placed proximal to the previous test site. The anesthetic concentration was increased (or decreased) in steps of 0.1% until the positive response disappeared (or *vice versa*). MAC was defined as the concentration midway between the highest concentration that permitted movement in response to the stimulus and the lowest concentration that prevented movement.

Tests of Locomotor Function

The effect of PSD-95/SAP90 or PSD-93/Chapsyn-110 antisense oligodeoxynucleotide on locomotor function was examined on the day after the last intrathecal oligodeoxynucleotide injection. The following tests were performed as described previously.[16,24] (1) Placing reflex: the mouse was held with the hind limbs slightly lower than the forelimbs, and the dorsal surface of the hind paws was brought into contact with the edge of a table. The experimenter recorded whether the mouse placed its hind paws on the table surface reflexively; (2) Grasping reflex: the mouse was placed on a wire grid, and the experimenter recorded whether the hind paws grasped the wire on contact; and (3) Righting reflex: the mouse was placed on its back on a flat surface, and the experimenter noted whether it immediately assumed the normal upright position. The scores for these reflexes were based on the counts of each normal reflex exhibited in six trials. To verify the effect of knockdown of the MAGUK proteins on the locomotor function of experimental animals, open field test was carried out in separate groups of mice according to the previous studies.[25,26] In brief, 24 h after the last intrathecal oligodeoxynucleotide injection, mice were placed in a special chamber (San Diego Instruments, San Diego, CA) to evaluate their locomotor activity for a 60-min period. A typical arena consists of a Plexiglas cage lined with infrared photo beams. A second frame with infrared photo beams is placed above the first one to measure rearing (*i.e.*, vertical activity). Movements including the activities in the center area of the chamber (central activity) and along the walls (thigmotaxis) of the chamber (peripheral activity) and vertical activity (rearing activity) are automatically recorded when a mouse breaks new photo beams. The size of the test arena was 15 inch × 15 inch × 12 inch. The number of beam breaking was counted for the three activities in each group.

Western Blot Analysis

Total protein from the lumbar enlargement segments of the spinal cords of mouse was extracted after MAC measurement. In separate groups of mice, the spinal cord was divided into the dorsal horn and the ventral horn by cutting straight across from the central canal laterally to a midpoint in the white matter.[17,27–29] The tissues were then homogenized in homogenization buffer[30] (10 mM Tris-HCl, 5 mM $MgCl_2$, 2 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 1 μM leupeptin, 2 μM pepstatin A, and 320 mM sucrose [pH 7.4]). The crude homogenates were centrifuged at 700*g* for 15 min at 4°C. The pellets were rehomogenized and spun again at 700*g*; then the supernatants were combined and diluted in resuspension buffer[30] (10 mM Tris-HCl, 5 mM $MgCl_2$, 2 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 1 μM leupeptin, 2 μM pepstatin A, and 250 mM sucrose [pH 7.4]). Next, the protein extracts were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrotransferred to the nitrocellulose membranes. The membranes were immunoblotted with affinity-purified mouse PSD-95/SAP90 antibody or polyclonal rabbit anti-PSD-93/Chapsyn-110 antibody diluted in blocking solution containing 3% nonfat dry milk and 0.1% Tween-20 in Tris-HCl-buffered saline for 1 h at room temperature. After being washed extensively, the membranes were incubated for 1 h with horseradish peroxidase-conjugated antimouse or antirabbit immunoglobulin (Bio-Rad Laboratories, Hercules, CA) at a dilution of 1:3,000. Specific proteins were detected by enhanced chemiluminescence (Amersham, Piscataway, NJ). Tubulin served as a loading control. The immunoblotting bands were quantified by densitometry using ImageJ software (National Institutes of Health, Bethesda, MD) and analyzed as described previously.[31,32] The data shown were representative of three independent experiments and the results after statistical analysis (figs. 1 and 2).

Statistical Analysis

Data are expressed as mean ± SEM. Statistical analysis was carried out with SigmaStat version 3.1 software (Systat Software Inc., Chicago, IL) by using one-way and two-way analysis of variance followed by Student-Newman-Keuls method. One-way analysis of variance was used to analyze between-group differences in WT mice, and two-way analysis of variance was used to analyze between-group differences in both WT and PSD-93/Chapsyn-110 knockout mice. The nonparametric test "Mann–Whitney U test" using SPSS version 13.0 software (SPSS Inc., Chicago, IL) was performed to determine the differences between saline group and other groups in tables 1 and 2. Statistical significance was set at $P < 0.05$ with use of a two-tailed analysis.

Results

Effect of Deficiency of PSD-95/SAP90 or PSD-93/Chapsyn-110 on Halothane MAC

By Western blotting, we verified that the protein levels of PSD-95/SAP90 and PSD-93/Chapsyn-110 in both the ven-

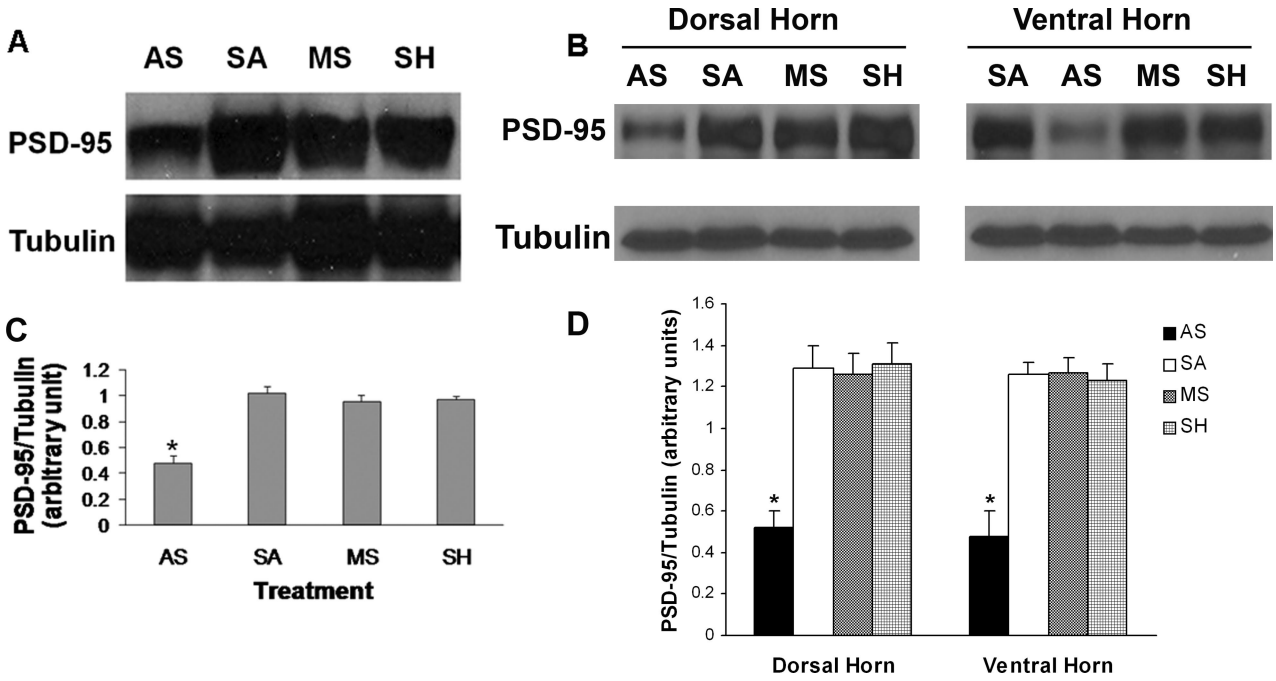

Fig. 1. The intrathecal injection of PSD-95/SAP90 antisense oligodeoxynucleotide (AS, 50 μg; n = 8) significantly inhibited PSD-95/SAP90 expression in both the ventral horn and the dorsal horn of the lumbar spinal cord. Neither missense (MS, 50 μg; n = 8) nor saline (SA; n = 6) injection had an effect. Tubulin served as a loading control. (*A*, *C*) Whole lumbar spinal cord; (*B*, *D*) the ventral horn and dorsal horn. No injection was given for sham (SH) rats (n = 6). The data shown are representative of three independent experiments (*A*, *B*) and the results after statistical analysis (*C*, *D*). * $P < 0.05$ *versus* SA group. PSD = postsynaptic density protein.

tral horn and the dorsal horn of the lumbar spinal cord were specifically decreased by intrathecal injection of their antisense oligodeoxynucleotides but not by their missense oligodeoxynucleotides (figs. 1 and 2). The intrathecal injection of PSD-95/SAP90 antisense (50 μg) inhibited PSD-95/SAP90 expression by 53% (fig. 1), and intrathecal injection of PSD-93/Chapsyn-110 antisense (12 μg) inhibited PSD-93/Chapsyn-110 expression by 50% (fig. 2). Knockdown of PSD-95/SAP90 or PSD-93/Chapsyn-110 dose-dependently and significantly reduced the threshold for halothane anesthesia compared with that of the saline-treated group (figs. 3 and 4). The intrathecal injection of PSD-95/SAP90 antisense oligodeoxynucleotide at different doses (25 and 50 μg) reduced halothane MAC of WT mice by 40 and 55%, respectively (fig. 4), and intrathecal injection of PSD-93/Chapsyn-110 antisense oligodeoxynucleotide at different doses (12 and 24 μg) reduced halothane MAC of WT mice by 25 and 53%, respectively (fig. 3). However, neither missense oligodeoxynucleotide had an effect on halothane MAC (figs. 3 and 4). Therefore, these antisense oligodeoxynucleotides are effective at inhibiting the expression and function of the respective proteins in the mice.

Effect of Combined PSD-95/SAP90 Knockdown with PSD-93/Chapsyn Deletion on Halothane MAC

Halothane MAC was measured in both WT and PSD-93/Chapsyn-110 knockout mice after intrathecal injections of PSD-95/SAP90 antisense oligodeoxynucleotide. We found that halothane MAC was similar in the two groups of mice. The intrathecal injection of PSD-95/SAP90 antisense oligodeoxynucleotide at different doses (25 and 50 μg) reduced halothane MAC of PSD-93/Chapsyn-110 knockout mice by 37 and 41%, respectively (fig. 4), and the effect was not significantly different from that in WT mice (fig. 4). These results suggest that combining PSD-95/SAP90 knockdown with PSD-93/Chapsyn-110 deletion does not have an additive effect on halothane MAC and that the functional role of PSD-95/SAP90 in halothane anesthesia is not enhanced after PSD-93/Chapsyn-110 deletion. As a control, intrathecal injection of PSD-93/Chapsyn-110 antisense (PSD-93 AS; 12 μg) or PSD-93/Chapsyn-110 missense (PSD-93 MS; 12 μg) oligodeoxynucleotide had no effect on halothane MAC in PSD-93 knockout mice (see Supplemental Digital Content 1, which is a figure showing the effect of PSD-98/Chapsyn-110 antisense oligodeoxynucleotide on halothane MAC in PSD-98 knockout mice, http://links.lww.com/ALN/A589).

Effect of Deficiency of PSD-95/SAP90 or PSD-93/Chapsyn-110 on Locomotor Function of Experimental Animals

Locomotor function testing showed that the knockdown of PSD-95/SAP90 or PSD-93/Chapsyn-110 had no significant influence on the animals' movements (tables 1 and 2; fig. 5). The scores for the three reflexes (placing, grasping, and righting) were not significantly different among different groups (tables 1 and 2). Open field test showed that the numbers of

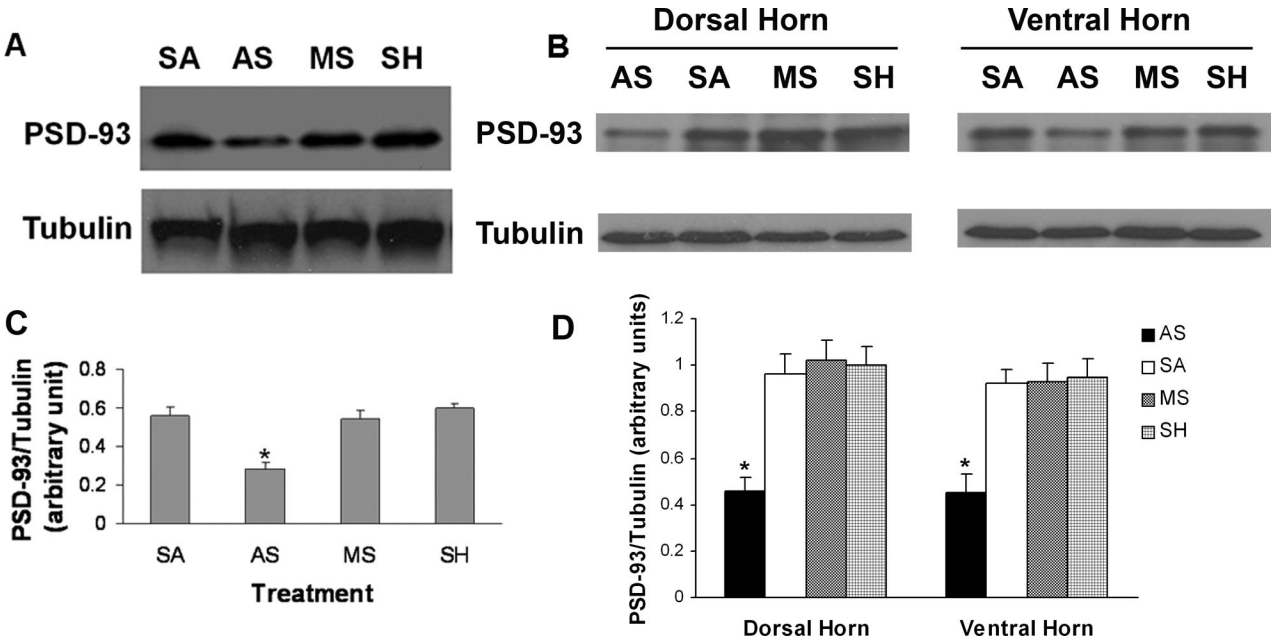

Fig. 2. Intrathecal injection of PSD-93/Chapsyn-110 antisense oligodeoxynucleotide (AS, 12 μg; n = 8) significantly inhibited PSD-93/Chapsyn-110 expression in both the ventral horn and the dorsal horn of the lumbar spinal cord. Neither missense (MS, 12 μg; n = 8) nor saline (SA; n = 6) injection had an effect. Tubulin served as a loading control. (*A*, *C*) Whole lumbar spinal cord; (*B*, *D*) the ventral horn and dorsal horn. No injection was given for sham (SH) rats (n = 6). The data shown are representative of three independent experiments (*A*, *B*) and the results after statistical analysis (*C*, *D*). * $P < 0.05$ *versus* saline group. PSD = postsynaptic density protein.

beam breaking for the three activities (central, peripheral, and rearing) were also not significantly different among different groups (fig. 5). Therefore, the intrathecal injection of these antisense oligodeoxynucleotides does not cause toxicity in the mice and is safe in this study.

Discussion

Our results indicate that (1) intrathecally injected antisense oligodeoxynucleotide of PSD-95/SAP90 or PSD-93/Chapsyn-110 significantly reduces halothane MAC; (2) halothane MAC is similar in WT and PSD-93/Chapsyn-110 knockout mice; and (3) the combination of PSD-95/SAP90 knockdown and PSD-93/Chapsyn-110 deletion does not have an additive effect on halothane anesthesia. These results suggest that PSD-95/SAP90 and PSD-93/Chapsyn-110 are involved in the molecular mechanisms of halothane anesthesia and that the functional role of PSD-95/SAP90 in halothane anesthesia is not enhanced after PSD-93/Chapsyn-110 deletion.

The PSD of excitatory synapses in the central nervous system is characterized by a dense network of proteins that include transmembrane receptors, scaffold proteins, and signaling molecules. Four members of the MAGUK family of scaffold proteins are abundantly expressed in the PSD. Each contains three PDZ domains, an Src homology 3 domain, and a catalytically inactive guanylate kinase domain, which together mediate protein–protein interactions important for channel clustering and recruitment of signaling complexes.[33] Of these MAGUK proteins, PSD-95/SAP90 and PSD-93/Chapsyn-110 are thought to have crucial roles in forming NMDA receptor-associated signaling complexes involved in

Table 1. PSD-95/SAP90 Knockdown Has No Effect on Locomotor Function of Unanesthetized Mice

Agent	Placing	Grasping	Righting
Saline (n = 8)	6 (0)	6 (0)	6 (0)
12.5 μg AS (n = 8)	6 (0)	6 (0)	6 (0)
25 μg AS (n = 8)	6 (0)	6 (0)	6 (0)
50 μg AS (n = 8)	5.75 (0.16)	6 (0)	6 (0)
50 μg MS (n = 8)	6 (0)	6 (0)	6 (0)

Data are expressed as mean (SEM) of six trials. Mice received a score of 1 for a normal response and 0 for failure.

AS = PSD-95 antisense oligodeoxynucleotide; MS = PSD-95 missense oligodeoxynucleotide; PSD = postsynaptic density protein.

Table 2. PSD-93/Chapsyn-110 Knockdown Has No Effect on Locomotor Function of Unanesthetized Mice

Agent	Placing	Grasping	Righting
Saline (n = 8)	6 (0)	6 (0)	5.75 (0.16)
6 μg AS (n = 8)	6 (0)	6 (0)	6 (0)
12 μg AS (n = 8)	6 (0)	6 (0)	5.75 (0.16)
24 μg AS (n = 8)	5.75 (0.16)	6 (0)	5.75 (0.16)
24 μg MS (n = 8)	5.75 (0.16)	6 (0)	6 (0)

Data are expressed as mean (SEM) of six trials. Mice received a score of 1 for a normal response and 0 for failure.

AS = PSD-93 antisense oligodeoxynucleotide; MS = PSD-93 missense oligodeoxynucleotide; PSD = postsynaptic density protein.

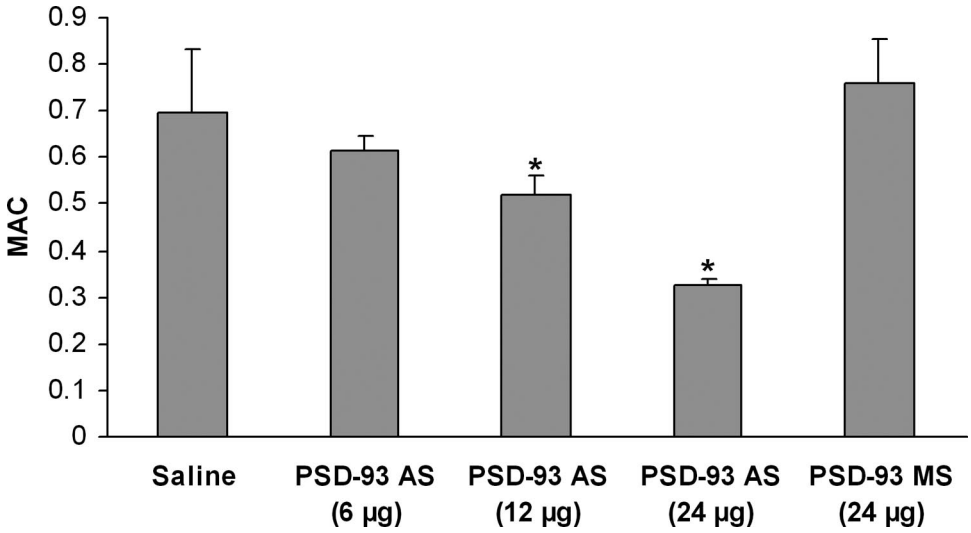

Fig. 3. The effect of PSD-93/Chapsyn-110 knockdown on halothane minimum alveolar concentration (MAC) in wild-type mice. The intrathecal injection of PSD-93/Chapsyn-110 antisense oligodeoxynucleotide (AS) dose-dependently reduced halothane MAC. However, missense injection had no effect. AS, n = 8 for each dose; missense oligodeoxynucleotide (MS), n = 8. * $P <$ 0.05 *versus* saline group (n = 6). PSD = postsynaptic density protein.

synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD).[34–37] Recently, Carlisle *et al.*[37] uncovered distinct roles for the two proteins in the hippocampal LTP and LTD by examining the plasticity phenotype in PSD-93/Chapsyn-110 and PSD-95/SAP90 mutant mice. Although LTP induction was facilitated and LTD was disrupted in the hippocampal CA1 region of PSD-95/SAP90 mutant mice, PSD-93/Chapsyn-110 mutant mice exhibited deficits in LTP and normal LTD in the hippocampus.[37] These results indicate that PSD-93/Chapsyn-110 and PSD-95/SAP90 have essentially opposite roles in the hippocampal LTP and LTD, perhaps because the two MAGUK proteins form distinct NMDA receptor signaling complexes that differentially regulate the induction of LTP and LTD by different patterns of synaptic activity in the hippocampus. MAGUK proteins also interact with transmembrane α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor regulatory proteins, which play a pivotal role in α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor trafficking at excitatory synapses.[38] α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor-mediated basal synaptic transmission is impaired in PSD-95/SAP90 mutant mice but normal in PSD-93/Chapsyn-110 mutant mice.[37]

In our previous studies, we showed that clinically relevant concentrations of inhalational anesthetics dose-dependently and specifically inhibit the PDZ domain-mediated protein interaction between NMDA receptors and PSD-95/SAP90 or PSD-93/Chapsyn-110.[18] However, it is unknown whether the two MAGUK proteins are simple substitutes for one another or they have separate regulatory mechanisms or sites of action in regard to anesthetic MAC. In the current study, we used a combination of genetic knockout and anti-

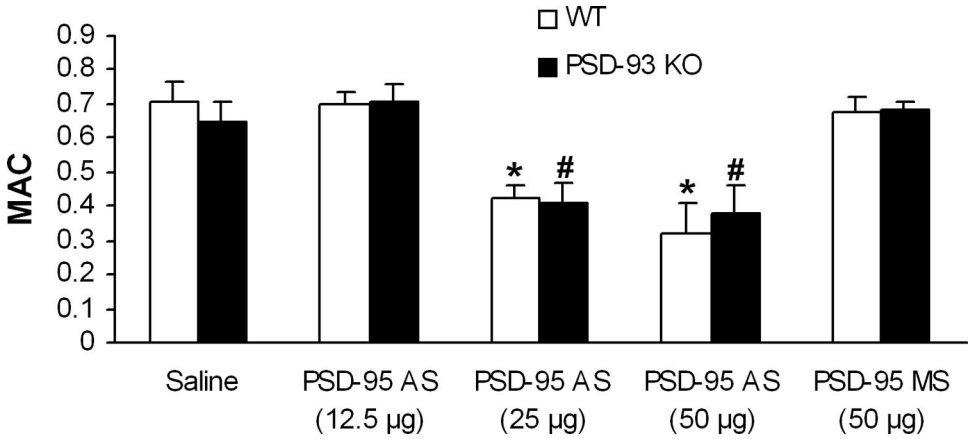

Fig. 4. Effect of PSD-95/SAP90 knockdown on halothane minimum alveolar concentration (MAC) in wild-type (WT) and PSD-93/Chapsyn-110 knockout (KO) mice. The intrathecal injection of PSD-95/SAP90 antisense oligodeoxynucleotide (AS) reduced halothane MAC in both WT and PSD-93/Chapsyn-110 KO mice at a similar level. AS, n = 8 for each dose; missense oligodeoxynucleotide (MS), n = 8. * $P <$ 0.05 *versus* saline group (n = 6) in WT mice and # $P <$ 0.05 *versus* saline group (n = 6) in PSD-93/Chapsyn-110 KO mice. PSD = postsynaptic density protein.

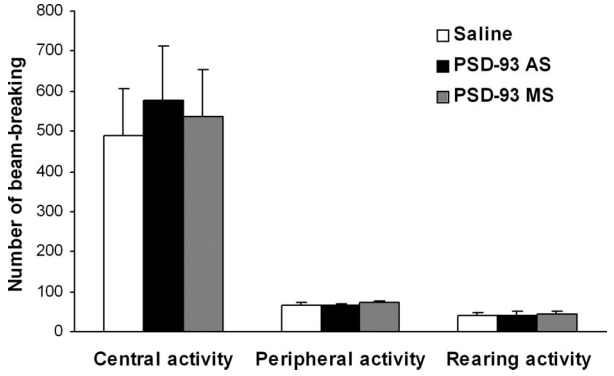

Fig. 5. The effect of PSD-93/Chapsyn-110 knockdown on locomotor function of wild-type mice in open-field test. The intrathecal injection of PSD-93/Chapsyn-110 antisense oligodeoxynucleotide (AS) had no effect on animal movements. The numbers of beam breaking in each activity were not significantly different in the three groups ($P > 0.05$ *vs.* saline group; n = 6). AS, n = 6; missense oligodeoxynucleotide (MS), n = 6. PSD = postsynaptic density protein.

sense oligodeoxynucleotide knockdown strategies to investigate the effect of deficiency of PSD-95/SAP90 and/or PSD-93/Chapsyn-110 on the threshold for halothane anesthesia. The significance of this study is to determine whether the two MAGUK proteins have additive effects on halothane anesthesia. Our data indicate that the spinal knockdown of PSD-95/SAP90 or PSD-93/Chapsyn-110 individually can reduce halothane MAC but combining spinal PSD-95/SAP90 knockdown with PSD-93/Chapsyn-110 deletion does not have an additive effect. Interestingly, a high dose (50 μg) of PSD-95/SAP90 antisense oligodeoxynucleotide had even less effect on halothane MAC in PSD-93/Chapsyn-110 knockout mice than that in WT mice. These results suggest that PSD-95/SAP90 and PSD-93/Chapsyn-110 contribute to halothane anesthesia and that the functional role of PSD-95/SAP90 in halothane anesthesia is not enhanced after PSD-93/Chapsyn-110 deletion. However, our current data cannot define whether the two MAGUK proteins act through distinct or the same signaling pathway in the molecular mechanisms of halothane anesthesia. We also found that halothane MAC was similar in WT and PSD-93/Chapsyn-110 knockout mice, implying that the role of PSD-93/Chapsyn-110 in halothane anesthesia can be compensated for in the knockout mice. Given that the effect of spinal PSD-95/SAP90 knockdown on halothane MAC was not enhanced in PSD-93/Chapsyn-110 knockout mice, it is unlikely that PSD-95/SAP90 compensates for PSD-93/Chapsyn-110, at least in regard to halothane anesthesia.

Previous studies have shown that the PSD-95/SAP90 and PSD-93/Chapsyn-110 are expressed in the whole spinal cord including ventral horn and dorsal horn.[17,39] In this study, we found that the intrathecal injection of PSD-95/SAP90 or PSD-93/Chapsyn-110 antisense oligodeoxynucleotide reduced the expression level of the respective protein in both the ventral horn and the dorsal horn of the spinal cord. Compared with the dorsal horn, the ventral horn expressed the two proteins in lower levels (62% for PSD-95/SAP90 and 48% for PSD-93/Chapsyn-110). However, our current data showed that antisense oligodeoxynucleotides knocked down the respective protein in a similar extent for the dorsal horn and ventral horn. Because the immobilizing properties of halothane take place in the spinal cord (mainly in the ventral horn[40] although halothane has some effect on the dorsal horn neurons), our results indicate that the knockdown of the two MAGUK proteins in both the ventral horn and/or the dorsal horn of the spinal cord contributes to halothane's immobilizing properties and might be actually involved in anesthetic mechanism. PSD-95/SAP90 and PSD-93/Chapsyn-110 have a subtle distinction in their expression profiles.[27,41] In the spinal cord, NMDA receptor subunits NR2A and NR2B also show different distribution.[42] It is possible that PSD-95/SAP90 and PSD-93/Chapsyn-110 predominantly associate with NMDA receptor complexes containing different NR2 subunits. If such segregation of MAGUK proteins into different complexes is substantiated by experiments, it could represent a feasible basis by which different MAGUK proteins contribute to different signaling processes involved in the molecular mechanisms of inhalational anesthesia.

In conclusion, our current study indicates that the two MAGUK proteins, PSD-95/SAP90 and PSD-93/Chapsyn-110, contribute to halothane anesthesia, that PSD-95/SAP90 does not compensate for PSD-93/Chapsyn-110 deletion, and that the functional role of PSD-95/SAP90 in halothane anesthesia is not enhanced after PSD-93/Chapsyn-110 deletion.

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