Effect of Xenon on Excitatory and Inhibitory Transmission in Rat Spinal Ventral Horn Neurons

Tomohiro Yamamoto, M.D.,* Hiroyuki Honda, M.D.,* Hiroshi Baba, M.D., Ph.D.,†
Tatsuro Kohno, M.D., Ph.D.‡

ABSTRACT

Background: The minimum alveolar concentration is determined in the spinal cord rather than in the brain. Xenon inhibits glutamatergic excitatory synaptic transmission in the dorsal horn neurons. However, its actions in the ventral horn neurons have not been investigated.

Methods: The effects of 50 or 75% xenon on excitatory and inhibitory synaptic transmission were examined in the spinal lamina IX neurons of neonatal rats by using a whole cell patch clamp technique.

Results: Fifty percent xenon inhibited the α-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid-induced currents (amplitudes = 72 ± 9% and integrated area = 73 ± 13% of the control values), and α-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid receptor-mediated electrically evoked excitatory postsynaptic currents (amplitudes = 69 ± 13% of the control values). Seventy-five percent xenon similarly inhibited α-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid-induced currents. However, xenon had no effect on the *N*-methyl-D-aspartate-induced currents or *N*-methyl-D-aspartate receptor-mediated electrically evoked excitatory postsynaptic currents. Xenon decreased the amplitude, but not the frequency, of miniature excitatory postsynaptic currents. There were no discernible effects on the currents induced by γ-aminobutyric acid or glycine or on miniature inhibitory postsynaptic currents.

Conclusions: Xenon inhibits α-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid receptor-mediated glutamatergic excitatory transmission in the spinal lamina IX neurons *via* a postsynaptic mechanism. In contrast, there are no substantial effects on *N*-methyl-D-aspartate receptor-mediated or inhibitory synaptic transmission. The suppressive effects on excitatory synaptic transmission in the ventral horn neurons partly account for the mechanism behind xenon's ability to produce immobility in response to noxious stimuli and to determine the minimum alveolar concentration.

What We Already Know about This Topic

- The site of action of anesthetics to prevent movement during surgery largely resides in the spinal cord
- Xenon produces anesthesia in part by reducing *N*-methyl-D-aspartate (NMDA) receptor and α-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid (AMPA) receptor activation, but whether it does so in the ventral horn is not known

What This Article Tells Us That Is New

- In the lamina IX neurons of rat spinal cord, xenon inhibits excitation of AMPA receptors but not NDMA receptors
- The immobilizing effects of xenon may be through an unexpected mechanism, reduction in excitation of AMPA receptors

XENON, as an inhalational anesthetic, combines profound anesthetic and analgesic properties with a low side-effect profile, and if used with an elaborate low-flow delivery system, it has the potential to become a main-line anesthetic.[1,2] Significant progress has been made toward elucidating how xenon produces anesthesia, and it has been revealed that xenon suppresses the excitatory synaptic transmission in the central nervous system.[3–8] A study on cultures of rat hippocampal neurons showed that xenon inhibited *N*-methyl-D-aspartate (NMDA) receptor-mediated synaptic transmission but not α-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid (AMPA) receptor-mediated synaptic transmission.[4] In later studies, xenon blocked both NMDA receptor-mediated and AMPA receptor-mediated synaptic transmission in cultures of mouse embryonic cortical neurons,[5] rodent amygdala,[6] prefrontal cortex,[7] and spinal cord dorsal horn.[7,8] By contrast, xenon had no effect on inhibitory synaptic transmission.[6–8]

Originally, the brain was considered to be the principal effect site of anesthetics and the determiner of the minimum alveolar concentration (MAC) for volatile anesthetics, a common means to express the strength or potency of anesthetics.

* Graduate Student, † Professor, ‡ Associate Professor, Division of Anesthesiology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan.

Received from the Division of Anesthesiology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan. Submitted for publication August 21, 2011. Accepted for publication January 25, 2012. Supported by Grants-in-Aid for Scientific Research (20390414, 21791438) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, Tokyo, Japan.

Address correspondence to Dr. Kohno: Division of Anesthesiology, Niigata University Graduate School of Medical and Dental Sciences, 1-757 Asahimachi, Chuo ku, Niigata 951-8510, Japan. kohno-t@umin.net. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

Copyright © 2012, the American Society of Anesthesiologists, Inc. Lippincott Williams & Wilkins. Anesthesiology 2012; 116:1025-34

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/116/5/1025/257830/0000542-201205000-00017.pdf by guest on 20 April 2024

However, the MAC merely refers to the anesthetic concentration needed to prevent movement in response to noxious stimuli[9] and is caused by anesthetic actions in the spinal cord, rather than in the brain.[10,11]

In the spinal cord, anesthetic actions might result from a combination of a reduction of the sensory transmission of nociceptive signals within the spinal dorsal horn and a suppression of the neuronal activity in the spinal ventral horn.[12] We reported previously that 50% xenon inhibited both NMDA receptor-medicated and AMPA receptor-mediated glutamatergic excitatory transmission *via* a postsynaptic mechanism in the spinal dorsal horn[8]; however, xenon's actions in the spinal ventral horn have not yet been clarified. Therefore, the current study investigated the effects of xenon on spinal ventral horn neurons.

Materials and Methods

All experimental procedures involving the use of animals were approved by the Animal Care and Use Committee at Niigata University Graduate School of Medical and Dental Sciences (Niigata, Japan).

Preparation of the Spinal Cord Slices

Neonatal Wistar rats (200 rats, 6–14 days old) were anesthetized with urethane (1.5 g/kg, intraperitoneal). Dorsal laminectomy was performed, and the lumbosacral segment of the spinal cord was removed.[13] The rats were killed immediately by exsanguination. Each spinal cord was placed in preoxygenated ice-cold artificial cerebrospinal fluid containing 117 mM NaCl, 3.6 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 25 mM $NaHCO_3$, and 11.5 mM D-glucose. After all the ventral and dorsal roots were cut, the pia-arachnoid membrane was removed. The spinal cord was mounted on the metal stage of a microslicer (DTK-1500; Dosaka, Kyoto, Japan) and cut into 500-μm transverse slices. Each spinal cord slice was transferred to a recording chamber and placed on the stage of an upright microscope equipped with an infrared-differential interference contrast system (E600FN; Nikon, Tokyo, Japan). The slice was fixed with an anchor and superfused at 4–6 ml/min with artificial cerebrospinal fluid equilibrated with a gas mixture of 95% O_2 and 5% CO_2 (pH = 7.4) and maintained at 36°C using a temperature controller (TC-324B; Warner Instruments, Hamden, CT).

Patch Clamp Recordings from Spinal Lamina IX Neurons

Lamina regions were identified under low magnification (×5 objective lens), and individual neurons were identified using a ×40 objective lens under an infrared-differential interference contrast microscope and monitored by a charge-coupled device camera (C2400–79H; Hamamatsu Photonics, Hamamatsu, Japan) on a video screen. Whole cell patch clamp recordings were made from the large lamina IX neurons (size, 15–25 μm).[13] In a previous study, these neurons were identified as motoneurons by fluorescence labeling with Evans blue dye injected into the rat hind limb the day before sacrifice.[14] Whole cell patch pipettes were constructed from borosilicate glass capillaries (1.5 mm OD; World Precision Instruments, Sarasota, FL). The resistance of a typical patch pipette was 4–8 MΩ when filled with internal solution. The patch pipette solutions contained 110 mM Cs_2SO_4, 5 mM tetraethylammonium, 0.5 mM $CaCl_2$, 2 mM $MgCl_2$, 5 mM EGTA, 5 mM HEPES, and 5 mM ATP-Mg.

Signals were amplified by an Axopatch 200B amplifier (Molecular Devices, Union City, CA), filtered at 2 kHz, and digitized at 5 kHz. All experiments were performed in voltage-clamp mode at a holding potential of −70 mV for recording exogenously applied AMPA-induced currents or AMPA receptor-mediated electrically evoked excitatory postsynaptic current (EPSCs) and miniature EPSCs. Exogenously applied NMDA-induced currents and NMDA receptor-mediated electrically evoked EPSCs were recorded at +40 mV. Inhibitory currents induced by exogenous γ-aminobutyric acid (GABA) or glycine, as well as miniature inhibitory postsynaptic current (IPSCs), were recorded at 0 mV. Electrically evoked EPSCs were elicited by focal stimulation of the deep dorsal horn with a concentric bipolar tungsten electrode (diameter = 10 μm). This stimulation area was identified visually under low magnification (×5 objective lens). Stimulus parameters were as follows: intensity, 1–5 V; duration, 0.05 ms; and frequency, 0.1 Hz. Electrically evoked EPSCs that displayed a constant latency and lack of failures with high frequency stimulation (10 Hz) were classified as monosynaptic. Data were stored and analyzed using a pCLAMP 9.2 data acquisition program (Molecular Devices) and Mini-analysis 6.0.7 (Synaptosoft, Leonia, NJ). Each peak current was measured before and after xenon treatment and expressed as (posttreatment/pretreatment) × 100 (as percentages).

Drug Application

Xenon was acquired from TG Showa (Tokyo, Japan). AMPA, NMDA, GABA, glycine, bicuculline, strychnine, 6-cyano-7-nitroquinoxaline-2,3-dione disodium, and D, L-2-amino-5-phosphonopentanoic acid were acquired from Sigma–Aldrich (St. Louis, MO). Tetrodotoxin was acquired from Wako (Osaka, Japan). Drugs were applied to the whole slice by perfusion *via* a three-way stopcock without changing the perfusion rate or temperature. The volume of the recording chamber was approximately 1.2 ml. The drugs reached the recording chamber within 15 s after the stopcock was opened, and the drugs were completely washed out within 30 s after the stopcock was closed. Xenon was mixed in a 1:1 ratio with a prefabricated gas containing 90% O_2 and 10% CO_2, so that the composition of the gas mixture was 50% xenon, 45% O_2, and 5% CO_2. For the additional experiments, xenon was mixed in a 3:1 ratio with a prefabricated gas containing 80% O_2 and 20% CO_2, so that the composition of the gas mixture was 75% xenon, 20% O_2, and 5% CO_2. Xenon was delivered through an ultrathin polyethyl-

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/116/5/1025/257830/0000542-201205000-00017.pdf by guest on 20 April 2024

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

ene tube and applied by bubbling through the perfusing solution saturated with the gas mixture for 30 min.

Statistical Analyses

Numerical data are represented as means ± SD. Statistical significance was determined as $P < 0.01$ using the Student paired *t* test or the Kolmogorov-Smirnov test, as appropriate. When referring to electrophysiological data, "n" indicates the number of neurons studied. The continuous curve for the concentration-response relationship of AMPA, NMDA, GABA, and glycine was drawn according to the Hill equation:

$$y = \frac{y_{max}x^n}{x^n - k^n}$$

where x is the agonist concentration, y is the relative amplitude of agonist-induced current (%), and y_{max} is the maximal value of y. The term k is the half-maximal effective concentration (EC_{50}; μM or mM), and n is the slope of the curve (Hill coefficient).

Results

EC_{50} of AMPA, NMDA, GABA, and Glycine on Spinal Lamina IX Neurons

Because there was no database available regarding the proper concentration of the agonists for the experiments in the spinal lamina IX neurons, the concentration-response curve was obtained for the agonists AMPA, NMDA, GABA, and glycine on the spinal lamina IX neurons (fig. 1). Exogenous application of AMPA elicited an inward current in neurons at −70 mV. Exogenous application of NMDA at +40 mV or of GABA or glycine at 0 mV elicited an outward current. Each agonist was applied by perfusion for 15 s and induced a current in a concentration-dependent manner. The EC_{50} values for AMPA, NMDA, GABA, and glycine on the spinal lamina IX neurons were as follows: AMPA, 7.0 μM; NMDA, 62.8 μM; GABA, 0.1 mM; and glycine, 0.2 mM. The Hill coefficient values were as follows: AMPA, 1.6; NMDA, 1.7; GABA, 1.1; glycine, 1.3. We set the concentration of the agonists as follows: AMPA, 10 μM; NMDA, 50 μM; GABA, 0.5 mM; glycine, 0.5 mM.

To confirm that these exogenous agonist-induced currents were postsynaptic phenomena, we examined these currents in the presence of tetrodotoxin (1 μM) to remove any possible influence of presynaptic neurons. Tetrodotoxin did not affect the amplitudes or the integrated area: AMPA (amplitude = 105 ± 12% of control values, $P = 0.36$; area = 106 ± 13% of control values, $P = 0.31$, n = 6), NMDA (amplitude = 95 ± 13% of control values, $P = 0.29$; area = 102 ± 23% of control values, $P = 0.84$, n = 9), GABA (amplitude = 108 ± 13% of control values, $P = 0.21$; area = 113 ± 12% of control values, $P = 0.05$, n = 5), glycine (amplitude = 110 ± 14% of control values, $P = 0.10$; area = 105 ± 18% of control values, $P = 0.50$, n = 6). Therefore, the following experiments to examine the effects of xenon on exogenous agonist-induced currents were performed in the absence of tetrodotoxin.

Effects of Xenon on Exogenous AMPA- and NMDA-induced Currents

Exogenous application of AMPA (10 μM, 15 s) elicited an inward current in the spinal lamina IX neurons at −70 mV (fig. 2A and D). Preapplication of 50% xenon for 5 min reversibly reduced the peak amplitudes of the AMPA-induced currents to 72 ± 9% of the control values (n = 7, $P <$ 0.01; fig. 2B) and the integrated area to 73 ± 13% of the control values (n = 7, $P < 0.01$; fig. 2C). Exogenous application of NMDA (50 μM, 15 s) induced an outward current at +40 mV (fig. 3A); 50% xenon did not change the peak amplitudes (99 ± 13% of control values, n = 6, $P = 0.91$) or the integrated area (99 ± 22% of control values, n = 6, $P =$ 0.91) of NMDA-induced currents.

Seventy-five percent xenon reversibly reduced the peak amplitudes of the AMPA-induced currents to 67 ± 12% of the control values (n = 10, $P < 0.01$; fig. 2E) and the integrated area to 62 ± 12% of the control values (n = 10, $P < 0.01$; fig. 2F). However, 75% xenon did not change the peak amplitudes (103 ± 7% of control values, n = 10, $P =$ 0.30) or the integrated area (100 ± 14% of control values, n = 10, $P = 0.97$) of NMDA-induced currents.

Effects of Xenon on AMPA and NMDA Receptor-mediated Electrically Evoked EPSCs

To examine the effects of xenon on synaptic transmission, electrically evoked EPSCs were elicited by focal stimulation of the deep dorsal horn. Evoked EPSCs can be divided into different groups. EPSCs that displayed a constant latency and lack of failure with high frequency stimulation (10 Hz) were classified as monosynaptic, whereas EPSCs that displayed variable latencies and failures were considered polysynaptic (fig. 4, A–C). We investigated the effects of xenon on evoked monosynaptic EPSCs.

α-Amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid receptor-mediated electrically evoked EPSCs were isolated pharmacologically with D,L-2-amino-5-phosphonopentanoic acid (50 μM), bicuculline (10 μM), and strychnine (2 μM) at −70 mV. Figure 5A shows the representative average traces of AMPA receptor-mediated evoked EPSCs before, during, and after xenon application. Fifty percent xenon reversibly reduced the peak amplitudes of AMPA receptor-mediated evoked EPSCs to 69 ± 13% of the control values (n = 9, $P < 0.01$). These evoked EPSCs were almost completely abolished by the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione disodium (10 μM, 10 ± 3% of the control values, n = 9, $P < 0.01$; fig. 5, A–C), suggesting that the evoked EPSCs were mediated by AMPA receptor.

N-methyl-D-aspartate receptor-mediated electrically evoked EPSCs were isolated pharmacologically with 6-cyano-7-nitroquinoxaline-2,3-dione disodium (10 μM), bicuculline (10

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/116/5/1025/257830/0000542-201205000-00017.pdf by guest on 20 April 2024

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

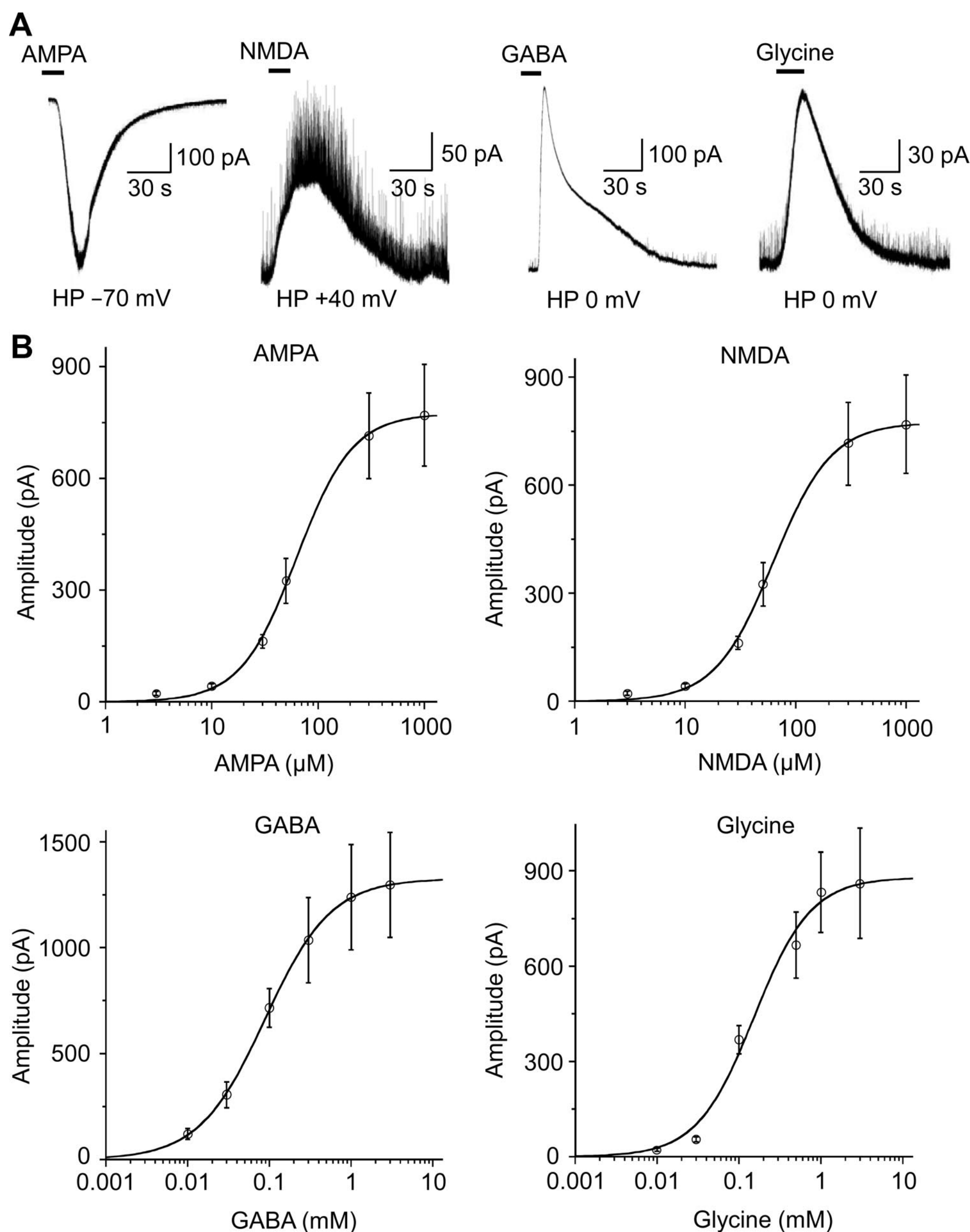

Fig. 1. Representative traces of agonist-induced currents and the concentration-response curve for each agonist. In this figure and subsequent figures, the *horizontal bars* in the recording charts indicate the duration of drug superfusion. Each agonist was applied for 15 s. Exogenous application of α-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid (AMPA) elicited an inward current in the spinal lamina IX neurons at −70 mV. Exogenous application of *N*-methyl-D-aspartate (NMDA) at +40 mV, γ-aminobutyric acid (GABA) at 0 mV, and glycine at 0 mV elicited an outward current (*A*). Peak amplitudes of the induced currents at various concentrations are plotted at the logarithm of their concentrations. Each *point with vertical bars* represents the mean value and SD. The continuous curves are drawn according to the Hill plot with each EC_{50} value and Hill coefficient. The EC_{50} values for AMPA, NMDA, GABA, and glycine on the spinal lamina IX neurons were as follows: AMPA 7.0 μM, NMDA 62.8 μM, GABA 0.1 mM, glycine 0.2 mM. Hill coefficient values for AMPA, NMDA, GABA, and glycine were as follows: AMPA 1.6, NMDA 1.7, GABA 1.1, glycine 1.3 (*B*). HP = holding potential.

μM), and strychnine (2 μM) at +40 mV. Figure 5D shows the representative average traces of NMDA receptor-mediated evoked EPSCs before and during xenon application. The peak amplitudes of NMDA receptor-mediated evoked EPSCs were not affected by 50% xenon (98 ± 3% of the control values, n = 6, P = 0.09). These evoked EPSCs were almost completely abolished by the NMDA receptor antagonist D,L-2-amino-5-phosphonopentanoic acid (50 μM, 7 ± 3% of the control values, n = 6, $P < 0.01$; fig. 5, D–F), suggesting that the evoked EPSCs were mediated by NMDA receptor.

Effects of Xenon on Miniature EPSCs

To determine the site of the inhibitory action of xenon, we examined the effects of xenon on miniature EPSCs in the

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/116/5/1025/257830/0000542-201205000-00017.pdf by guest on 20 April 2024

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

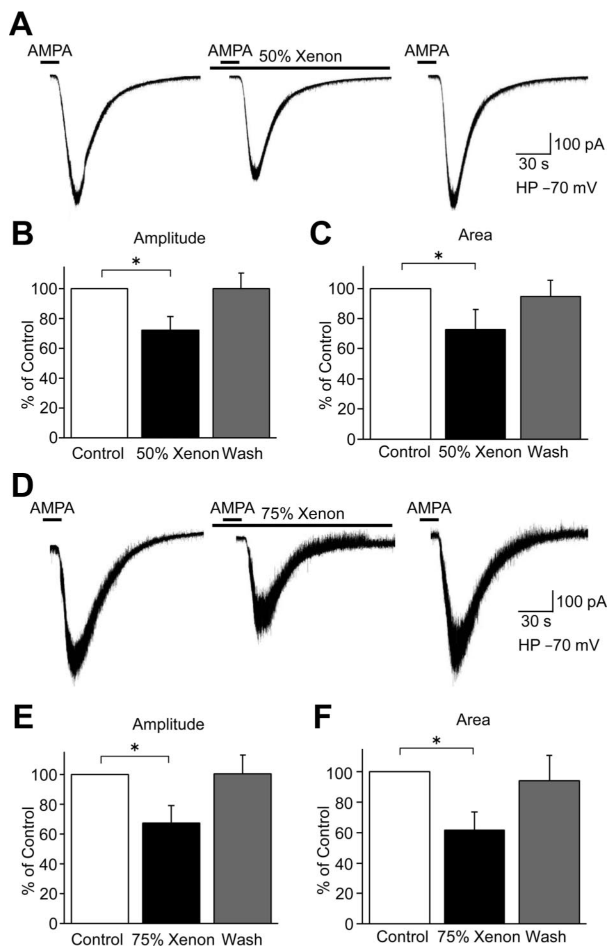

Fig. 2. Xenon inhibited exogenous α-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid (AMPA)-induced currents. Representative traces showing AMPA (10 μM)-induced currents in the spinal lamina IX neurons at −70 mV, which were reversibly reduced by 50% xenon (*A*). Fifty percent xenon decreased the peak amplitudes of AMPA-induced currents to 72 ± 9% of control values and the integrated area to 73 ± 13% of control values, respectively (*B, C*). Representative traces showing AMPA-induced currents in the spinal lamina IX neurons at −70 mV, which were reversibly reduced by 75% xenon (*D*). Seventy-five percent xenon decreased the peak amplitudes of AMPA-induced currents to 67 ± 12% of control values and the integrated area to 62 ± 12% of control values (*E, F*). * $P < 0.01$. HP = holding potential.

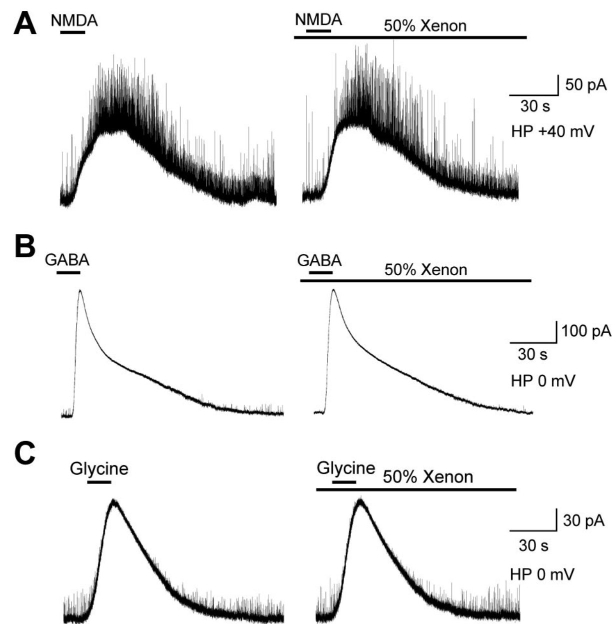

Fig. 3. Xenon did not affect exogenous *N*-methyl-D-aspartate (NMDA)-, γ-aminobutyric acid (GABA)-, and glycine-induced currents. Representative traces showing NMDA (50 μM)-induced currents in the spinal lamina IX neurons at + 40 mV. Fifty percent xenon did not change the peak amplitudes and the integrated area of NMDA-induced currents (*A*). Representative traces showing current induced by GABA (0.5 mM) in the spinal lamina IX neurons at 0 mV. Fifty percent xenon did not change the peak amplitudes and the integrated area of GABA-induced currents (*B*). Representative traces showing currents induced by glycine (0.5 mM) in the spinal lamina IX neurons at 0 mV. Fifty percent xenon did not change the peak amplitudes and the integrated area of glycine-induced currents (*C*). HP = holding potential.

presence of tetrodotoxin (1 μM) at −70 mV. Fifty percent xenon decreased the mean amplitudes of the miniature EPSCs to 82 ± 14% of the control values (n = 9, $P < 0.01$), whereas the mean frequencies of miniature EPSCs remained unchanged (102 ± 40% of the control values, n = 9, $P = 0.91$, fig. 6A). Figure 6B shows the effects of xenon on cumulative distributions of miniature EPSC amplitudes and interevent intervals. Although xenon increased the proportion of miniature EPSCs with a significantly smaller amplitude compared with that of the control ($P < 0.01$), it had no effect on the cumulative distribution of the interevent intervals of miniature EPSCs ($P = 0.39$). The decay time of miniature EPSCs was reversibly prolonged by xenon (111 ± 25% of the control values, n = 9, $P = 0.22$); however, this change was not statistically significant.

Effects of Xenon on Exogenous GABA- and Glycine-induced Currents and on Miniature IPSCs

Exogenous application of GABA (0.5 mM, 15 s) and glycine (0.5 mM, 15 s) elicited an outward current at 0 mV (fig. 3, B and C). Fifty percent xenon did not change the peak amplitudes (99 ± 9% of the control values, n = 10, $P = 0.76$) or the integrated area (101 ± 19% of the control values, n = 10, $P = 0.87$) of GABA-induced currents. Similarly, xenon did not change the peak amplitudes (105 ± 13% of the control values, n = 9, $P = 0.29$) or the integrated area (108 ± 12% of the control values, n = 9, $P = 0.07$) of glycine-induced currents.

We also examined the effects of xenon on miniature IPSCs in the presence of tetrodotoxin (1 μM) at 0 mV. Fifty percent xenon did not change the mean amplitudes (100 ± 32% of the control values, n = 9, $P = 0.97$; fig. 7A) or the mean frequencies (100 ± 20% of the control values, n = 9, $P = 0.95$) of miniature IPSCs. Xenon had no effect on the cumulative distributions of miniature IPSC amplitudes ($P = 0.15$; fig. 7B) or interevent intervals ($P = 0.61$).

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/116/5/1025/257830/0000542-201205000-00017.pdf by guest on 20 April 2024

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

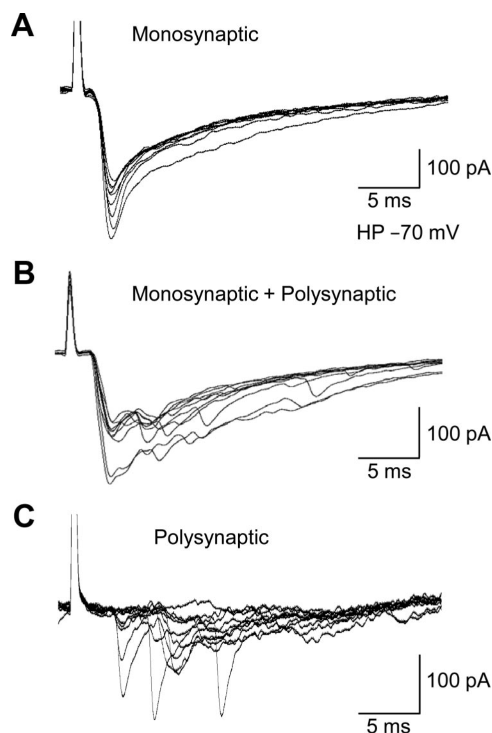

Fig. 4. Various patterns of excitatory postsynaptic currents (EPSCs) elicited by focal stimulation at a frequency of 10 Hz. Monosynaptic evoked EPSCs (*A*). Both the monosynaptic- and polysynaptic-evoked EPSCs (*B*). Polysynaptic evoked EPSCs (*C*). These evoked EPSCs were observed in different neurons. HP = holding potential.

Discussion

Several lines of evidence suggest that xenon suppresses excitatory synaptic transmission in central nervous system areas such as the brain and spinal cord dorsal horn.[3–8] However, its actions in the spinal ventral horn have not been clarified. Thus, we investigated the effects of xenon on excitatory and inhibitory synaptic transmission in the spinal ventral horn neurons.

In the current study, 50 and 75% xenon inhibited both the peak amplitudes and the integrated area of exogenous AMPA-induced currents, as well as the peak amplitudes of AMPA receptor-mediated evoked EPSCs. In addition, 50% xenon decreased the mean amplitudes of miniature EPSCs, but the mean frequency of miniature EPSCs remained unchanged. However, 50 and 75% xenon had no substantial effect on exogenous NMDA-induced currents or NMDA receptor-mediated evoked EPSCs. In addition, 50% xenon had no effect on currents induced by GABA or glycine or on miniature IPSCs. Taken together, these findings suggest that xenon acts on both synaptic and extrasynaptic AMPA receptors and inhibits AMPA receptor-mediated glutamatergic excitatory transmission *via* a postsynaptic mechanism in the spinal lamina IX neurons.

However, several studies have demonstrated that xenon depressed both NMDA and AMPA receptor-mediated glutamatergic excitatory synaptic transmission postsynaptically in the amygdala,[6] prefrontal cortex,[7] and substantia gelatinosa,[7,8] whereas xenon did not affect inhibitory synaptic

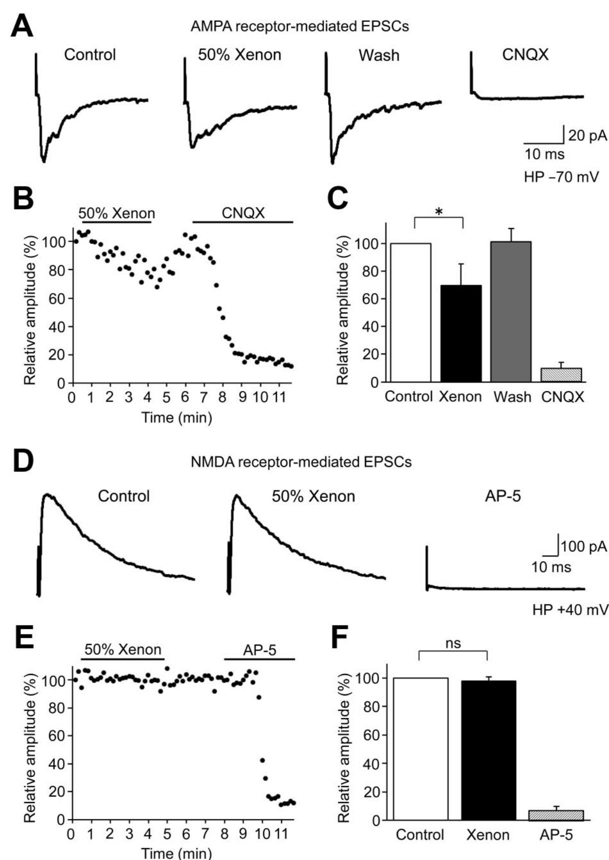

Fig. 5. Xenon reversibly decreased the amplitudes of α-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid (AMPA) receptor-mediated electrically evoked excitatory postsynaptic currents (EPSCs) but not *N*-methyl-D-aspartate (NMDA) receptor-mediated electrically evoked EPSCs in the spinal lamina IX neurons. Averaged traces of six consecutive AMPA receptor-mediated evoked EPSCs at −70 mV before, during, and after xenon (*A*). Time course of the relative peak amplitudes of AMPA receptor-mediated evoked EPSCs before, during, and after xenon (*B*). Summaries of the suppressive effects of xenon and 6-cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX) on AMPA receptor-mediated evoked EPSCs relative to control value (*C*). Averaged traces of six consecutive NMDA receptor-mediated evoked EPSCs at +40 mV before and during xenon (*D*). Time course of the relative peak amplitudes of NMDA receptor-mediated evoked EPSCs before and during xenon (*E*). Summaries of the suppressive effects of xenon and D,L-2-amino-5-phosphonopentanoic acid (AP-5) on NMDA receptor-mediated evoked EPSCs relative to control value (*F*). $* P < 0.01$. The time scales of electrically evoked EPSCs are 1,000 times as small as those of exogenous agonist-induced currents. HP = holding potential; ns = not significant.

transmission. This discrepancy between our results and those of previous studies could be attributed to a different composition of NMDA receptors expressed in these neurons.

The NMDA receptor has been proposed as a major target for xenon.[4,15,16] This receptor is composed of subunits from at least two families: NR1 and NR2. The NR1 subunit is essential for the function of NMDA receptors and is ex-

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/116/5/1025/257830/0000542-201205000-00017.pdf by guest on 20 April 2024

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

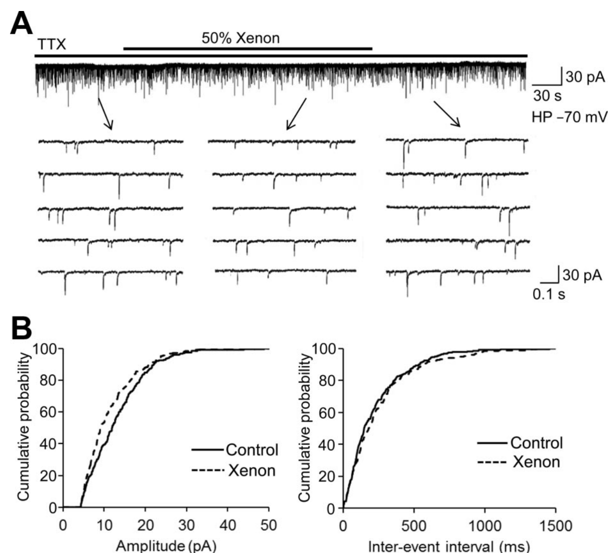

Fig. 6. Xenon decreased the mean amplitudes but not the mean frequency of miniature excitatory postsynaptic currents (EPSCs) in the spinal lamina IX neurons. Continuous chart recording of miniature EPSCs at −70 mV in the presence of Tetrodotoxin (TTX) (1 μM) during the action of xenon (*upper trace*). Five consecutive traces of miniature EPSCs are shown on an expanded time scale (*lower traces*) (*A*). Cumulative distributions of the amplitude (*left*) and interevent interval (*right*) of miniature EPSCs before (*continuous line*) and during (*dotted line*) the action of xenon. Xenon had little effect on the distribution of the interevent interval but shifted the distribution to a smaller amplitude, as determined using the Kolmogorov-Smirnov test (*B*). HP = holding potential.

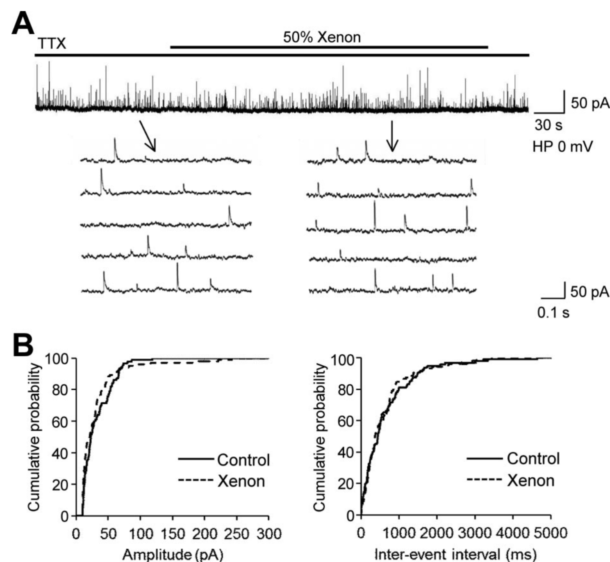

Fig. 7. Xenon affected neither the mean amplitudes nor the mean frequency of miniature inhibitory postsynaptic currents (IPSCs) in the spinal lamina IX neurons. Continuous chart recording of miniature IPSCs at 0 mV in the presence of Tetrodotoxin (TTX) (1 μM) during the action of xenon (*upper trace*) (*A*). Five consecutive traces of miniature IPSCs are shown on an expanded time scale (*lower traces*). Cumulative distributions of the amplitude (*left*) and interevent interval (*right*) of miniature IPSCs before (*continuous line*) and during (*dotted line*) the action of xenon. Xenon had little effect on the distributions both of the amplitude and interevent interval, as determined using the Kolmogorov-Smirnov test (*B*). HP = holding potential.

pressed ubiquitously in the entire spinal cord, including the spinal ventral horn motoneurons and the spinal dorsal horn neurons.[17–20] The functional properties of NMDA receptors are determined by the NR2 subunit composition (NR2A–2D).[20–22] The NR3 subunits (NR3A and 3B) occasionally are expressed in addition to NR1 and NR2 subunits and functionally suppress NMDA receptors.[23,24] The NR2C and NR2D subunits are expressed weakly, but the NR2A and NR2B subunits are not identified in substantia gelatinosa neurons of the adult rat lumbar spinal cord.[18] We reported previously that 50% xenon depressed NMDA receptor-mediated glutamatergic excitatory transmission *via* a postsynaptic mechanism in adult substantia gelatinosa neurons.[8] By contrast, the NR2A subunit is expressed predominantly in neonatal motoneurons.[19] However, around postnatal day 10–14, the NR2A subunit decreases, and the major regulatory subunit of the NMDA receptors switches to the NR3B subunit; the NR3B subunit is maintained until the adult stage.[19] The expression of the NR3B subunit is restricted to somatic motoneurons in the adult brainstem and spinal cord.[19] Considering the neonatal age of rats in the current study, these reports suggest that we may have examined the effects of xenon mainly on the NR2A subunit; the lack of NMDA receptor response to xenon may be caused by the lack of response of the NR2A subunit to xenon. In short, the subunits of the NMDA receptors expressed in neonatal motoneurons and in adult substantia gelatinosa neurons are different. This different composition of NMDA receptors may underlie the variability among tissues in the effects of xenon on NMDA receptor-mediated synaptic transmission.

In our current study, we were confronted with the issue of xenon concentration. At the beginning, a single concentration of 50% xenon was used in our experiments for technical and financial reasons. The MAC of xenon is estimated to be 86–161% in rats[25,26]; thus, 50% xenon is calculated to be 0.31–0.58 rat MAC. Xenon is known to have analgesic properties even at subanesthetic (0.3 MAC) concentrations in rats[27] and humans.[28,29] Although the concentration of dissolved xenon could not be measured, we calculated the concentration of dissolved 50% xenon at approximately 1.9 mM according to previous studies.[4–6,30,31] A previous study showed that 1.9 mM dissolved xenon depressed the NR2A-containing NMDA receptors in the amygdala.[32] In addition, we previously demonstrated that 50% xenon depressed NMDA receptor-mediated glutamatergic excitatory transmission in adult substantia gelatinosa neurons.[8] Therefore, we concluded that this 50% xenon concentration was sufficient to modulate synaptic transmission in the spinal lamina IX neurons. Nevertheless, 50% xenon did not affect NMDA

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/116/5/1025/257830/0000542-201205000-00017.pdf by guest on 20 April 2024

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

receptor-mediated synaptic transmission in our current investigation. However, xenon may suppress NMDA receptor-mediated synaptic transmission or may produce some other effects at higher concentration. These effects would be more critical to immobilization than the effects on AMPA receptor-mediated synaptic transmission observed in this study. Thus, we also examined the effects of 75% xenon on AMPA- and NMDA-induced currents. Seventy-five percent xenon reversibly reduced the peak amplitudes and the integrated area of the AMPA-induced currents. However, 75% xenon did not change the peak amplitudes or the integrated area of NMDA-induced currents. Considering the maintenance of physiologic concentration of oxygen and carbon dioxide, 75% xenon is the maximum concentration in our current investigation. Nevertheless, NMDA-induced currents were not affected by 75% xenon.

Non-NMDA glutamate receptors include both AMPA receptors and kainate receptors. Neonatal rat motoneurons also express kainate receptors.[33] However, most of the glutamatergic non-NMDA receptor-mediated synaptic transmissions in neonatal rodent motoneurons are mediated *via* AMPA receptors.[34–36] Therefore, we interpreted glutamatergic non-NMDA receptor-mediated synaptic transmission as AMPA receptor-mediated synaptic transmission in this study.

The effects of 50% xenon on AMPA receptor-mediated synaptic transmission in the spinal lamina IX neurons were less than those in the spinal dorsal horn neurons[8]; however, several previous reports have indicated that the dorsal horn plays a lesser role in immobility and have suggested that the ventral horn is the site of anesthetic actions.[37–39] These results might also suggest that xenon acts differently than other anesthetics by acting predominantly on the spinal dorsal horn neurons, rather than on the spinal ventral horn motoneurons.

In a previous study, Cheng and Kendig[35] investigated the effects of the inhalational anesthetic enflurane on synaptic transmission in spinal ventral horn neurons. Electrically evoked EPSCs were elicited by electrical stimuli applied to the dorsal root, and the researchers concluded that enflurane depressed glutamatergic excitatory synaptic transmission in motoneurons.[35] However, the inhalational anesthetic isoflurane[40] and nitrous oxide[41] affected synaptic transmission in substantia gelatinosa neurons of the adult rat spinal cord. It was also reported that isoflurane affected electrically evoked EPSCs in substantia gelatinosa neurons of the immature rat spinal cord.[42] In addition, xenon inhibited AMPA and NMDA receptor-mediated excitatory synaptic transmission in substantia gelatinosa neurons of the adult rat spinal cord.[7,8] These results suggest that the electrically evoked postsynaptic currents may be affected in the substantia gelatinosa before reaching the spinal lamina IX neurons when the dorsal root or root entry zone is stimulated. Thus, in the current study, we elicited electrically evoked EPSCs by focal stimulation of the deep dorsal horn. Despite using this method, we could not completely exclude the possibility that deep dorsal horn stimulation might evoke monosynaptic responses such as Ia inputs that may not be relevant to the MAC determination.

The ventral interneuronal networks are also important for generating motor responses to noxious stimuli and determining the MAC.[37,39] The importance of ventral interneurons is supported by the evidence that spinal sensory neurons and motoneurons are relatively resistant to anesthetics, despite showing some individually depressive effects.[43]

To identify large spinal lamina IX neurons under an infrared-differential interference contrast microscope, we excluded older rats, whose spinal lamina IX neurons were impossible to identify because of highly developed fibrous tissue. However, the major regulatory subunit of the NMDA receptors switches from NR2A to NR3B in motoneurons around postnatal day 10–14,[19] and this switch of NMDA receptor subunits indicates a possibility that xenon may depress NMDA receptor-mediated synaptic transmission in adult motoneurons. In contrast, another study showed that the sensitivities of NMDA receptors to Mg^{2+}, isoflurane, ketamine, nitrous oxide, and ethanol were not altered by the NR3B subunit, although the NR3B subunit prominently reduced the amplitude of NMDA currents.[44] These results indicate that NMDA receptor-mediated synaptic transmission may not be affected by xenon in adult motoneurons. In any case, additional studies are required using identified spinal motoneurons in the adult rat.

In conclusion, we investigated the effects of xenon on the spinal lamina IX neurons as one of the components of the spinal reflex pathway and clarified that xenon inhibited AMPA receptor-mediated glutamatergic excitatory synaptic transmission in the spinal lamina IX neurons *via* a postsynaptic mechanism. These results indicate that the suppression of neuronal activity in the spinal ventral horn and substantia gelatinosa neurons might contribute to immobility and could account for the mechanism of xenon as an anesthetic for the prevention of movement in response to noxious stimuli and to determine the MAC. However, the modest effects of xenon on AMPA receptors and the lack of effect on the NMDA receptors might mean that the spinal ventral horn neurons are not a likely site for the immobilizing action of xenon; additional investigation of ventral interneurons existing in the lamina VII and VIII would help resolve these questions.

References

1. Preckel B, Schlack W: Inert gases as the future inhalational anaesthetics? Best Pract Res Clin Anaesthesiol 2005; 19: 365–79
2. Derwall M, Coburn M, Rex S, Hein M, Rossaint R, Fries M: Xenon: Recent developments and future perspectives. Minerva Anestesiol 2009; 75:37–45

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/116/5/1025/257830/0000542-201205000-00017.pdf by guest on 20 April 2024

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

3. Preckel B, Weber NC, Sanders RD, Maze M, Schlack W: Molecular mechanisms transducing the anesthetic, analgesic, and organ-protective actions of xenon. ANESTHESIOLOGY 2006; 105:187–97
4. de Sousa SL, Dickinson R, Lieb WR, Franks NP: Contrasting synaptic actions of the inhalational general anesthetics isoflurane and xenon. ANESTHESIOLOGY 2000; 92:1055–66
5. Dinse A, Föhr KJ, Georgieff M, Beyer C, Bulling A, Weigt HU: Xenon reduces glutamate-, AMPA-, and kainate-induced membrane currents in cortical neurones. Br J Anaesth 2005; 94:479–85
6. Haseneder R, Kratzer S, Kochs E, Eckle VS, Zieglgänsberger W, Rammes G: Xenon reduces *N*-methyl-D-aspartate and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor-mediated synaptic transmission in the amygdala. ANESTHESIOLOGY 2008; 109:998–1006
7. Haseneder R, Kratzer S, Kochs E, Mattusch C, Eder M, Rammes G: Xenon attenuates excitatory synaptic transmission in the rodent prefrontal cortex and spinal cord dorsal horn. ANESTHESIOLOGY 2009; 111:1297–307
8. Georgiev SK, Furue H, Baba H, Kohno T: Xenon inhibits excitatory but not inhibitory transmission in rat spinal cord dorsal horn neurons. Mol Pain 2010; 6:25
9. Eger EI 2nd, Saidman LJ, Brandstater B: Minimum alveolar anesthetic concentration: A standard of anesthetic potency. ANESTHESIOLOGY 1965; 26:756–63
10. Antognini JF, Schwartz K: Exaggerated anesthetic requirements in the preferentially anesthetized brain. ANESTHESIOLOGY 1993; 79:1244–9
11. Rampil IJ, Mason P, Singh H: Anesthetic potency (MAC) is independent of forebrain structures in the rat. ANESTHESIOLOGY 1993; 78:707–12
12. Collins JG, Kendig JJ, Mason P: Anesthetic actions within the spinal cord: Contributions to the state of general anesthesia. Trends Neurosci 1995; 18:549–53
13. Honda H, Baba H, Kohno T: Electrophysiological analysis of vulnerability to experimental ischemia in neonatal rat spinal ventral horn neurons. Neurosci Lett 2011; 494:161–4
14. Wang MY, Rampil IJ, Kendig JJ: Ethanol directly depresses AMPA and NMDA glutamate currents in spinal cord motor neurons independent of actions on GABAA or glycine receptors. J Pharmacol Exp Ther 1999; 290:362–7
15. Franks NP, Dickinson R, de Sousa SL, Hall AC, Lieb WR: How does xenon produce anaesthesia? Nature 1998; 396:324
16. Yamakura T, Harris RA: Effects of gaseous anesthetics nitrous oxide and xenon on ligand-gated ion channels. Comparison with isoflurane and ethanol. ANESTHESIOLOGY 2000; 93:1095–101
17. Kutsuwada T, Kashiwabuchi N, Mori H, Sakimura K, Kushiya E, Araki K, Meguro H, Masaki H, Kumanishi T, Arakawa M, Mishina M: Molecular diversity of the NMDA receptor channel. Nature 1992; 358:36–41
18. Tölle TR, Berthele A, Zieglgänsberger W, Seeburg PH, Wisden W: The differential expression of 16 NMDA and non-NMDA receptor subunits in the rat spinal cord and in periaqueductal gray. J Neurosci 1993; 13:5009–28
19. Fukaya M, Hayashi Y, Watanabe M: NR2 to NR3B subunit switchover of NMDA receptors in early postnatal motoneurons. Eur J Neurosci 2005; 21:1432–6
20. Ishii T, Moriyoshi K, Sugihara H, Sakurada K, Kadotani H, Yokoi M, Akazawa C, Shigemoto R, Mizuno N, Masu M: Molecular characterization of the family of the *N*-methyl-D-aspartate receptor subunits. J Biol Chem 1993; 268:2836–43
21. Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH: Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron 1994; 12:529–40
22. Yamakura T, Shimoji K: Subunit- and site-specific pharmacology of the NMDA receptor channel. Prog Neurobiol 1999; 59:279–98
23. Nishi M, Hinds H, Lu HP, Kawata M, Hayashi Y: Motoneuron-specific expression of NR3B, a novel NMDA-type glutamate receptor subunit that works in a dominant-negative manner. J Neurosci 2001; 21:RC185
24. Sucher NJ, Akbarian S, Chi CL, Leclerc CL, Awobuluyi M, Deitcher DL, Wu MK, Yuan JP, Jones EG, Lipton SA: Developmental and regional expression pattern of a novel NMDA receptor-like subunit (NMDAR-L) in the rodent brain. J Neurosci 1995; 15:6509–20
25. David HN, Leveille F, Chazalviel L, MacKenzie ET, Buisson A, Lemaire M, Abraini JH: Reduction of ischemic brain damage by nitrous oxide and xenon. J Cereb Blood Flow Metab 2003; 23:1168–73
26. Koblin DD, Fang Z, Eger EI 2nd, Laster MJ, Gong D, Ionescu P, Halsey MJ, Trudell JR: Minimum alveolar concentrations of noble gases, nitrogen, and sulfur hexafluoride in rats: Helium and neon as nonimmobilizers (nonanesthetics). Anesth Analg 1998; 87:419–24
27. Watanabe I, Takenoshita M, Sawada T, Uchida I, Mashimo T: Xenon suppresses nociceptive reflex in newborn rat spinal cord *in vitro*: Comparison with nitrous oxide. Eur J Pharmacol 2004; 496:71–6
28. Yagi M, Mashimo T, Kawaguchi T, Yoshiya I: Analgesic and hypnotic effects of subanaesthetic concentrations of xenon in human volunteers: Comparison with nitrous oxide. Br J Anaesth 1995; 74:670–3
29. Petersen-Felix S, Luginbühl M, Schnider TW, Curatolo M, Arendt-Nielsen L, Zbinden AM: Comparison of the analgesic potency of xenon and nitrous oxide in humans evaluated by experimental pain. Br J Anaesth 1998; 81:742–7
30. Hapfelmeier G, Zieglgänsberger W, Haseneder R, Schneck H, Kochs E: Nitrous oxide and xenon increase the efficacy of GABA at recombinant mammalian GABA(A) receptors. Anesth Analg 2000; 91:1542–9
31. Plested AJ, Wildman SS, Lieb WR, Franks NP: Determinants of the sensitivity of AMPA receptors to xenon. ANESTHESIOLOGY 2004; 100:347–58
32. Haseneder R, Kratzer S, Kochs E, Höfelmann D, Auberson Y, Eder M, Rammes G: The xenon-mediated antagonism against the NMDA receptor is non-selective for receptors containing either NR2A or NR2B subunits in the mouse amygdala. Eur J Pharmacol 2009; 619:33–7
33. Stegenga SL, Kalb RG: Developmental regulation of *N*-methyl-D-aspartate- and kainate-type glutamate receptor expression in the rat spinal cord. Neuroscience 2001; 105:499–507
34. Cheng G, Kendig JJ: Enflurane decreases glutamate neurotransmission to spinal cord motor neurons by both pre- and postsynaptic actions. Anesth Analg 2003; 96:1354–9
35. Cheng G, Kendig JJ: Enflurane directly depresses glutamate AMPA and NMDA currents in mouse spinal cord motor neurons independent of actions on GABAA or glycine receptors. ANESTHESIOLOGY 2000; 93:1075–84
36. Li HF, Wang MY, Knape J, Kendig JJ: Ethanol tachyphylaxis in spinal cord motorneurons: Role of metabotropic glutamate receptors. Br J Pharmacol 2003; 138:1417–24
37. Jinks SL, Martin JT, Carstens E, Jung SW, Antognini JF: Peri-MAC depression of a nociceptive withdrawal reflex is accompanied by reduced dorsal horn activity with halothane but not isoflurane. ANESTHESIOLOGY 2003; 98:1128–38
38. Kim J, Yao A, Atherley R, Carstens E, Jinks SL, Antognini JF: Neurons in the ventral spinal cord are more depressed by isoflurane, halothane, and propofol than are neurons in the dorsal spinal cord. Anesth Analg 2007; 105:1020–6
39. Mitsuyo T, Dutton RC, Antognini JF, Carstens E: The differential effects of halothane and isoflurane on windup of

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/116/5/1025/257830/0000542-201205000-00017.pdf by guest on 20 April 2024

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

dorsal horn neurons selected in unanesthetized decerebrated rats. Anesth Analg 2006; 103:753–60

40. Wakai A, Kohno T, Yamakura T, Okamoto M, Ataka T, Baba H: Action of isoflurane on the substantia gelatinosa neurons of the adult rat spinal cord. ANESTHESIOLOGY 2005; 102: 379–86
41. Georgiev SK, Kohno T, Ikoma M, Yamakura T, Baba H: Nitrous oxide inhibits glutamatergic transmission in spinal dorsal horn neurons. Pain 2008; 134:24–31
42. Haseneder R, Kurz J, Dodt HU, Kochs E, Zieglgänsberger W, Scheller M, Rammes G, Hapfelmeier G: Isoflurane reduces glutamatergic transmission in neurons in the spinal cord superficial dorsal horn: Evidence for a presynaptic site of an analgesic action. Anesth Analg 2004; 98:1718–23
43. Eger EI 2nd, Raines DE, Shafer SL, Hemmings HC Jr, Sonner JM: Is a new paradigm needed to explain how inhaled anesthetics produce immobility? Anesth Analg 2008; 107: 832–48
44. Yamakura T, Askalany AR, Petrenko AB, Kohno T, Baba H, Sakimura K: The NR3B subunit does not alter the anesthetic sensitivities of recombinant *N*-methyl-D-aspartate receptors. Anesth Analg 2005; 100:1687–92

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/116/5/1025/257830/0000542-201205000-00017.pdf by guest on 20 April 2024

ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

Weeding Out Piso's Cure

Originally formulated in 1864 with opium and morphine as "Piso's Consumption Cure," this glorified cough syrup shed its opiates by the 1880s. By 1904 "Piso's Cure" was renamed "Piso's Remedy for Coughs and Colds" (*left*). In May of 1909 the American Medical Association blacklisted "Piso's Cure" as a "habit-forming drug." Perhaps that is understandable, since "piso" (Spanish for "floor") was a marijuana-chloroform elixir which "floored" its imbibers. (Copyright © the American Society of Anesthesiologists, Inc.)

George S. Bause, M.D., M.P.H., Honorary Curator, ASA's Wood Library-Museum of Anesthesiology, Park Ridge, Illinois, and Clinical Associate Professor, Case Western Reserve University, Cleveland, Ohio. UJYC@aol.com.

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.