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Xenon suppresses nociceptive reflex in newborn rat spinal cord in vitro; comparison with nitrous oxide

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Abstract

Although analgesic action of xenon has been reported, little is known about the effect of xenon at the spinal cord, which plays a crucial role in nociceptive transmission. We studied the effect of xenon on nociceptive reflex (the slow ventral root potential) and the monosynaptic reflex in neonatal rat spinal cord in vitro in comparison with nitrous oxide. Xenon (30%) and nitrous oxide (30%) were applied for 17 min through superfusing artificial cerebrospinal fluid. Xenon and nitrous oxide significantly reduced the amplitude of nociceptive reflex by ~ 70% and ~ 25%, respectively. Xenon and nitrous oxide also significantly reduced the amplitude of the monosynaptic reflex by ~ 35% and ~ 15%, respectively. These results indicate that xenon suppressed the synaptic transmission at the spinal cord, especially those of the slow ventral root potential, which reflect nociceptive transmission.

Keywords: General anesthetic; Inhalation; Monosynaptic reflex; Nociceptive reflex; Slow ventral root potential; Neonatal spinal cord

1. Introduction

Because of its inert characteristic and its low solubility in the blood, xenon is gaining popularity as a general anesthetic in which analgesia and immobility to noxious stimuli are two of the most important features. While analgesic effect of another gaseous anesthetic nitrous oxide has long been recognized clinically, xenon was recently reported to have potent analgesic action in human (Petersen-Felix et al., 1998; Yagi et al., 1995). For both analgesia and immobility in anesthesia, the spinal cord plays a crucial role as the site of action of anesthetics (Kendig, 2002). The slow ventral root potential elicited by stimulating the dorsal root of the neonatal rat spinal cord in vitro is shown to be indicative of nociception (Otsuka and Konishi, 1974); it is elicited by noxious stimuli to the periphery, it can be elicited by electrical stimulation of dorsal roots at an intensity corresponding to the threshold of small diameter nociceptive afferents, it is suppressed by many analgesics including morphine (Woodley and Kendig, 1991). Furthermore, the early component of the slow ventral root potential is reported to be NMDA (*N*-methyl-D-aspartate) dependent and the late component to be substance P dependent (Woodley and Kendig, 1991). Recently, xenon and nitrous oxide have been shown to block the NMDA receptor (Franks et al., 1998; Jevtovic-Todorovic et al., 1998). As the NMDA receptor plays an important role in nociceptive transmission at the spinal cord (Randic et al., 1993), this inhibitory action of xenon and nitrous oxide on the NMDA receptor is postulated as one of the mechanisms of their analgesic action (Franks et al., 1998). The aim of the present study was to examine the effect of xenon on the slow ventral root potential in order to evaluate xenon's antinociceptive action at the spinal cord, in comparison with equipotent nitrous oxide.

2. Materials and methods

2.1. Spinal cord preparation and perfusion

The study protocol was approved by our institution's animal care committee. Neonatal (1–3 days old) SD rats were anesthetized with halothane, soaked in iced water for about 1 min, and decapitated. The spinal cord (from mid-thoracic to lower sacral) were rapidly removed, placed in a

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chamber (3.6 ml) and superfused with the artificial cerebrospinal fluid at the rate of 2–2.5 ml/min. All the tubing was made of polyethylene tube. The artificial cerebrospinal fluid was consisted of (in mM) NaCl 113, KCl 4.5, NaH_2PO_4 1, $NaHCO_3$ 25, $CaCl_2$ 2, $MgCl_2$ 1, glucose 11, and was equilibrated with 95% O_2 and 5% CO_2. Temperature was monitored using a fine thermistor probe positioned near the spinal cord. All experiments were done at 25 ± 0.5 °C.

2.2. Drug application and measurement

For xenon application, a closed circuit was employed between an artificial cerebrospinal fluid bubbling bottle and a plastic reservoir bag. A gas mixture of 95% xenon + 5% CO_2 (Kyodo Sanso, Wakayama, Japan) and a gas mixture of 95% O_2 + 5% CO_2 were fed to the closed circuit through precise flow meters to make up the gas mixture of 30% xenon + 65% O_2 + 5% CO_2. The xenon concentration of the closed circuit was continuously monitored by the heat conductivity method (XP-314, New Cosmos Electric, Osaka, Japan). For nitrous oxide application, a pre-made gas mixture of 30% nitrous oxide + 65% O_2 + 5% CO_2 (Sumitomo Fine Chemicals, Osaka, Japan) was bubbled in the artificial cerebrospinal fluid. Each gas mixture containing an anesthetic was bubbled for more than 30 min before use. To maintain the same oxygen concentration, 30% N_2 was used as the control for 30% xenon and 30% nitrous oxide. A fresh spinal cord preparation was used for each drug application.

2.3. Monosynaptic reflex and slow ventral root potential

A stimulating suction electrode was placed on a dorsal root of a spinal cord, and a recording suction electrode was placed on the corresponding ipsilateral ventral root at its exit from the spinal cord. To evoke the monosynaptic reflex and the slow ventral root potential, single square wave pulses (0.2 ms in duration), of the intensity well supramaximal for exciting the C-fiber (20–50 mA), were applied every 30 s. Those evoked potentials were amplified, low-pass filtered (DC to 3 kHz), digitized at a sampling rate of 10 kHz for the monosynaptic reflex and 100 Hz for the slow ventral root potential, and analyzed (MacLab, AD Instruments). Those responses were also recorded using a DAT recorder (PC200A, SONY, Tokyo) at the sampling rate of 20 kHz.

After recording the control response for 20 min, each anesthetic was applied to the spinal cord for 17 min, and was washed out for 20 min. Every 5 min, five consecutive responses were averaged. For example, the data expressed as ‘‘at 15 min’’ was the average of five consecutive responses from 14 to 16 min. The amplitude of the monosynaptic reflex and the slow ventral root potential were measured. For the slow ventral root potential, amplitude was measured at 0.5 and 3.0 s after the stimulation. The former represents early component of the slow ventral root potential and the latter represents late component of the slow ventral root potential.

2.4. Statistical analysis

Two-factor analysis of variance for repeated measures was used, and then Tukey test was performed. $P < 0.05$ was considered significant. Data were expressed as mean ± S.E.

3. Results

3.1. Effects of 30% xenon and 30% nitrous oxide on the monosynaptic reflex

In the xenon group, the control amplitude was 3.00 ± 0.07 mV ($n = 5$). The amplitude was significantly ($P < 0.01$) suppressed to 1.93 ± 0.70 mV after 15 min of application, but still not plateaued (Figs. 1A and 2). It

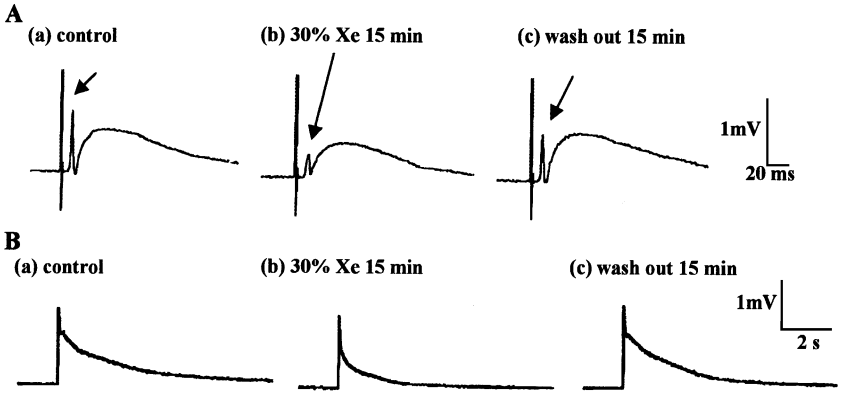

Fig. 1. Xenon’s effect on the monosynaptic reflex and the slow ventral root potential. (A) Typical recordings of the monosynaptic reflex (arrow) are shown. The monosynaptic reflexes follow stimulation artifacts. The monosynaptic reflex was suppressed by xenon and recovered. (B) Typical recordings of the slow ventral root potential are shown. The sharp upward spikes (which consist of the stimulation artifact and the monosynaptic reflex) are followed by the declining slow ventral root potentials lasting more than 10 s. The slow ventral root potential was suppressed by xenon and recovered. (a) control, (b) 15 min after the application of 30% xenon, (c) 15 min upon termination and wash out of xenon. Notice the difference in time scales.

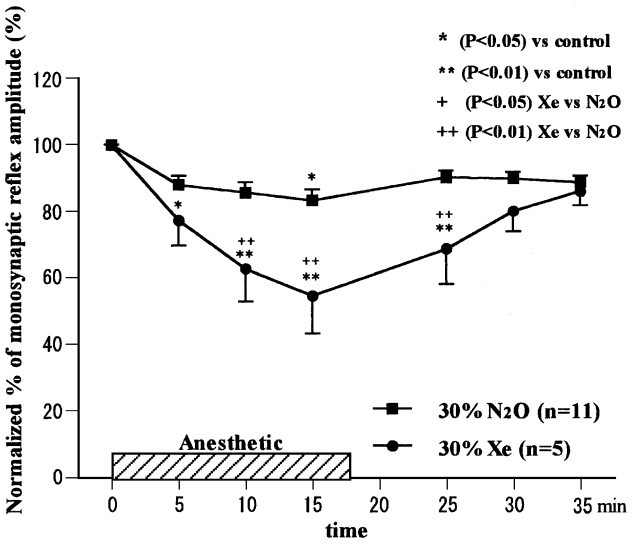

Fig. 2. Anesthetics' effect on the amplitude of the monosynaptic reflex. The amplitude of the monosynaptic reflex was significantly and reversibly suppressed by 30% xenon (circle, $n=5$) and 30% nitrous oxide (square, $n=11$). The mean and S.E. of normalized % are plotted and the application of anesthetics is indicated by a bar. Asterisks (*, **) indicate significant ($*P<0.05$, $**P<0.01$) changes compared to the respective control. Pluses (+, ++) indicate significant ($+P<0.05$, $++P<0.01$) difference between the effects of xenon and nitrous oxide at a given time.

recovered to 2.66 ± 0.74 mV at 15 min after washout. In the nitrous oxide group, the amplitude was 3.86 ± 0.08 mV ($n=11$) in control, suppressed to 3.25 ± 0.60 mV after 15 min of application, and 3.35 ± 0.57 mV at 15 min after washout. The suppression was significant ($P<0.05$), and almost plateaued after 15 min of application. These suppressions expressed in the form of normalized % are shown in Fig. 2. At 15 min of application, the suppression by xenon was significantly greater than that by nitrous oxide ($P<0.01$).

3.2. Effects of 30% xenon and 30% nitrous oxide on the early component of the slow ventral root potential

The amplitude of the slow ventral root potential at 0.5 s from the evoked stimulus was significantly and reversibly suppressed by two anesthetics. The control amplitude of 0.33 ± 0.07 mV ($n=5$) was significantly ($P<0.01$) suppressed to 0.12 ± 0.09 mV after 15 min of application of xenon, and recovered to 0.34 ± 0.16 mV at 15 min after washout (Figs. 1 and 3). With nitrous oxide, the amplitude was 0.80 ± 0.04 mV ($n=11$) in control, was significantly ($P<0.05$) suppressed to 0.62 ± 0.07 mV at 15 min after application, and recovered to 0.83 ± 0.10 mV at 15 min after washout. Although the effect of nitrous oxide reached the plateau within 5 to 10 min, the effect of xenon did not plateau at 15 min of application. These effects expressed in the form of normalized % are shown in Fig. 3. At 5, 10 and 15 min of application, the suppression by xenon was significantly greater than that by nitrous oxide ($P<0.01$).

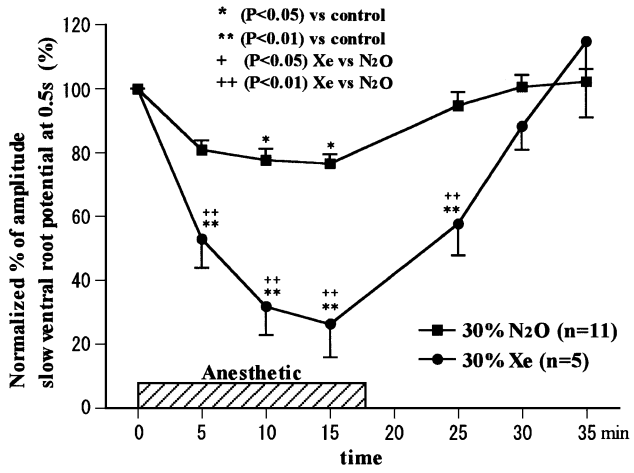

Fig. 3. Anesthetics' effect on the amplitude of the early component of the slow ventral root potential. The amplitude of the slow ventral root potential at 0.5 s from the evoked stimulus was significantly and reversibly suppressed by 30% xenon (circle, $n=5$) or 30% nitrous oxide (square, $n=11$). The mean and S.E. of normalized % are plotted and the application of anesthetics is indicated by a bar. Asterisks (*, **) indicate significant ($*P<0.05$, $**P<0.01$) changes compared to the respective control. Pluses (+, ++) indicate significant ($+P<0.05$, $++P<0.01$) difference between the effects of xenon and nitrous oxide at a given time.

3.3. Effects of 30% xenon and 30% nitrous oxide on the late component of the slow ventral root potential

The amplitude of the slow ventral root potential at 3.0 s from the evoked stimulus was also significantly and reversibly suppressed by two anesthetics (Figs. 1 and 4). The control amplitude of 0.18 ± 0.01 mV ($n=5$) was signifi-

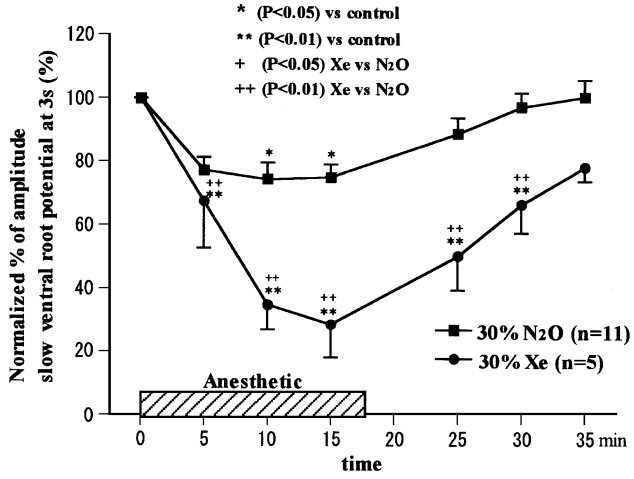

Fig. 4. Anesthetics' effect on the amplitude of the late component of the slow ventral root potential. The amplitude of the slow ventral root potential at 3 s from the evoked stimulus was significantly and reversibly suppressed by 30% xenon (circle, $n=5$) or 30% nitrous oxide (square, $n=11$). The mean and S.E. of normalized % are plotted and the application of anesthetics is indicated by a bar. Asterisks (*, **) indicate significant ($*P<0.05$, $**P<0.01$) changes compared to the respective control. Pluses (+, ++) indicate significant ($+P<0.05$, $++P<0.01$) difference between the effects of xenon and nitrous oxide at a given time.

cantly ($P<0.01$) suppressed to 0.05 ± 0.02 mV after 15 min of application of xenon, and recovered to 0.15 ± 0.06 mV at 15 min after washout. With nitrous oxide, the amplitude was 0.51 ± 0.01 mV ($n=11$) in control, was significantly ($P<0.05$) suppressed to 0.38 ± 0.05 mV after 15 min of application of nitrous oxide, and recovered to 0.52 ± 0.07 mV at 15 min after washout. Although the effect of nitrous oxide reached the plateau within 5 to 10 min, that of xenon barely reached the plateau at 15 min after application. These effects expressed in the form of normalized % are shown in Fig. 4. At 5, 10 and 15 min after application, the suppression by xenon was significantly greater than that by nitrous oxide ($P<0.01$).

4. Discussion

At the isolated spinal cord, xenon suppressed the synaptic transmission significantly, while another gaseous anesthetic nitrous oxide exerted only minimal effect.

4.1. Concentrations of anesthetics

Ostwald liquid/gas partition coefficients at 25 °C for xenon and nitrous oxide are 0.0885 and 0.55, respectively (Firestone et al., 1986). The concentrations of anesthetics in the artificial cerebrospinal fluid bubbled with 30% xenon and 30% nitrous oxide were calculated as 1.09 and 6.75 mM, respectively. Minimum alveolar concentration (MAC) has been widely used to indicate the potency of inhalation anesthetics. MAC is EC_{50} of anesthetics measuring escape movements against noxious stimuli. In adult rat, MAC of xenon and nitrous oxide are reported to be 161% and 136–204%, respectively (Koblin et al., 1998). Ostwald liquid/gas partition coefficient at 37 °C for xenon and nitrous oxide are 0.075 and 0.39, respectively (Firestone et al., 1986). Using these coefficients, MAC corresponding concentrations in the artificial cerebrospinal fluid at 37 °C for xenon and nitrous oxide were calculated as 4.75 and 20.8–31.3 mM, respectively. As anesthetic potencies in the aqueous-phase are relatively temperature-independent (Franks and Lieb, 1996), rough estimate is that 30% xenon and 30% nitrous oxide in the present study correspond to 0.23 and 0.22–0.32 MAC, respectively. However, as MAC in neonatal rat could be different from adult rat, and the exact concentrations of anesthetics of the perfusate in the recording chamber were not measured, there is certain limitation in evaluating the present results.

4.2. Effect on the monosynaptic reflex

Xenon had stronger effect than nitrous oxide. Thirty percent xenon depressed the monosynaptic reflex amplitude to 64% of the control, whereas 30% nitrous oxide suppressed to 84%. So far, there is no report about the effect of xenon on the monosynaptic reflex, but there are some about nitrous oxide. In spinalized cats, 50% nitrous oxide suppresses the monosynaptic reflex amplitude to 50–55% of the control value (De Jong et al., 1968). Also in spinalized cats, 33% nitrous oxide is shown to suppress the monosynaptic reflex amplitude to 57% of the control (Sugai et al., 1982). The discrepancy in potency of nitrous oxide may be due to the difference in preparations used (Fig. 2).

The monosynaptic reflex is a compound action potential, elicited by the dorsal root (presynapse) stimulation and recorded from the ventral root (postsynapse). The postsynaptic event includes the glutamate receptor reaction and the following excitation of motoneurons and ventral roots. Since the monosynaptic reflex is glutamate non-NMDA receptor origin (Long et al., 1990), and xenon is shown to have little effect on non-NMDA receptor current (de Sousa et al., 2000), then the suppression of the monosynaptic reflex observed here indicates that xenon should have inhibitory effect either on the presynaptic process or on the postsynaptic excitability or both. Nitrous oxide is reported to have no presynaptic effect but it suppresses non-NMDA receptor mildly (Mennerick et al., 1998). This suppression can account for the inhibitory effect of nitrous oxide on the monosynaptic reflex.

4.3. The slow ventral root potential may indirectly indicate the excitation of secondary afferent neurons and reflect the nociceptive transmission

4.3.1. Pathway of the slow ventral root potential

The slow ventral root potential is elicited by the dorsal root stimulation and recorded from the ventral root, but the pathway between the dorsal root and the motoneuron is unknown, monosynaptic and/or polysynaptic connections can be considered. Monosynaptic connection is suggested by the following facts (Otsuka and Yoshioka, 1993): (i) Dorsal rhizotomy results in a significant decrease in the substance P content of the ventral horn. (ii) Substance P-immunoreactive axonal buttons form synapses with dendrites of motoneurons in the spinal cord. (iii) The ionophoretic application of substance P to motoneurons induces a depolarization. These reports indicate that substance P-containing primary afferent fibers might directly, in other word monosynaptically, connect to motoneurons in the ventral horn. On the other hand, multi-synaptic connection is suggested by the following arguments: (i) As non-NMDA receptor is related to the fast synaptic event of monosynaptic connection, the slow synaptic event such as the slow ventral root potential should not be blocked by non-NMDA receptor antagonist. (ii) But non-NMDA receptor antagonist CNQX (6-Cyano-7-nitroquinoxaline-2,3-dione) depresses or almost abolishes the slow ventral root potential (Thompson et al., 1992). (iii) The firing of interneurons can be blocked by non-NMDA receptor antagonist. (iv) The suppressive effect of CNQX on the slow ventral root potential indicates the involvement of interneurons, accordingly multi-synaptic.

4.3.2. Implications of the slow ventral root potential

Because the ventral root potential represents the compound membrane potentials of overall motoneurons reacted to the dorsal root stimulation, the slow ventral root potential may reflect the excitation of secondary afferent neurons in nociceptive transmission. Indeed, the slow ventral root potential is considered to reflect the nociceptive neurotransmission by the following reasons (Kendig et al., 1991): (i) It can be evoked by nociceptive stimuli such as tail-pinching or electrical stimulation in a strength-dependent manner. (ii) It is inhibited by morphine, and its inhibition can be antagonized by naloxone. (iii) Its threshold is almost the same as that of C-fibers (Nussbaumer et al., 1989).

4.3.3. Early and late components of the slow ventral root potential

The slow ventral root potential is known to be the sum of two components, an early component and a late component. The early component is NMDA receptor origin, lasts for about 1.5 s, and is the main component of the slow ventral root potential during 0.1 to 1.0 s from the evoking stimulus. The late component is tachykinin (substance P) receptor origin, lasts for more than 10 s, and is the major component of the slow ventral root potential after 1.5 s from the stimulus (Nussbaumer et al., 1989; Woodley and Kendig, 1991). In the present study, we measured the amplitude of the slow ventral root potential after 0.5 and 3 s from the stimulus as the representative of the early component and the late component, respectively.

4.4. Antinociceptive action of xenon and nitrous oxide at the spinal cord was indicated by the suppression of the slow ventral root potential

In the present study, the amplitude of the early component of the slow ventral root potential was suppressed to 36% and 78% of the control after 15 min application of 30% xenon and 30% nitrous oxide, respectively. Likewise, the amplitude of the late component of the slow ventral root potential was suppressed to 27% and 75% of the control by xenon and nitrous oxide, respectively (Figs. 3 and 4).

These results indicate that xenon has a potent antinociceptive action at the spinal cord level, while nitrous oxide has mild one, both at low concentrations equivalent to 0.2–0.3 MAC. Suppression of nociceptive transmission by these anesthetics has been shown in animals as well as in humans. In cats, responses to noxious stimuli of wide dynamic range neurons in the dorsal horn of the spinal cord are suppressed equally by 70% xenon and 70% nitrous oxide (Utsumi et al., 1997). Also in human volunteers, low concentrations of xenon and nitrous oxide are reported to have the same analgesic effect (Petersen-Felix et al., 1998; Yagi et al., 1995). In the present study, there was a difference in the extent of the slow ventral root potential suppression between 30% xenon and 30% nitrous oxide, which are reported to have the same analgesic potency in vivo (Petersen-Felix et al., 1998; Yagi et al., 1995). This difference might arise from the spinal cord preparation itself. Antinociceptive action of nitrous oxide has been shown to depend on the descending inhibitory system (Zhang et al., 1999), so the action of nitrous oxide could be underestimated in the present spinal cord preparation, which lacked the descending inhibitory system. In a spinalized cat, which lacks the descending inhibitory system, wide dynamic range neuronal response to noxious stimuli is suppressed by xenon but not by nitrous oxide (Miyazaki et al., 1999). NMDA subtype of glutamate receptor has been shown to play an important role in pain transmission (Klepstad et al., 1990), and its role in wind-up phenomenon at the spinal cord is well established (Randic et al., 1993). Accordingly, NMDA receptor antagonists can exert antinociceptive action. Xenon (Franks et al., 1998) and nitrous oxide (Jevtovic-Todorovic et al., 1998; Mennerick et al., 1998) are shown to act as NMDA receptor antagonist using cultured cells or slice preparations, implying that these anesthetics could exert antinociceptive effect through their property as NMDA receptor antagonists. This can explain the suppression of the early component of the slow ventral potential by the anesthetics shown in the present study. Substance P is one of neurotransmitters released from the primary afferent C and Aδ fibers and play a distinct role in nociceptive transmission. Its receptor (neurokinin-1 receptor) antagonists have antinociceptive action (Henry, 1993). So the suppression of the late component of the slow ventral root potential by two anesthetics is also indicative of their antinociceptive action at the spinal cord. Since there is no report about the effect of general anesthetics on the action of substance P or on the tachykinin receptor, the precise mechanism of this inhibition of substance P-mediated slow component is not known. NMDA is reported to accelerate substance P release from the primary afferent nociceptors (Liu et al., 1997), and this may be part of the reason for the inhibition of substance P-mediated slow component by anesthetics. With all three potentials (the monosynaptic reflex, the early and the late component of the slow ventral root potential), nitrous oxide almost reached its maximal effect within 15 min, while xenon did not reached plateau phase even at 15 min of application (Figs. 3 and 4). This means that 30% xenon could be more potent if applied longer. In an in vivo study, however, the effect of inhaled xenon on spinal cord neurons reaches its maximum within 10 min (Utsumi et al., 1997). In addition, xenon is known for its shorter induction and emergence from general anesthesia compared to nitrous oxide due to its lower blood/gas partition coefficient. The reason for this discrepancy is not clear. It may be that the diffusion from the surface of the spinal cord takes longer time for xenon than the diffusion from the blood to the nervous tissue.

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