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MAC reduction after intrathecal coadministration of $GABA_A$ agonist and glutamate antagonist in rats

Hiroyuki Sumikura[1,2], and Lars Arendt-Nielsen[1]

[1] Department of Anesthesiology, St. Marianna University School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki 216-0015, Japan
[2] Center for Sensory-Motor Interaction, Department of Health Science and Technology, Aalborg University, Fredrik Bajers Vej 7 D-3, DK-9220 Aalborg, Denmark

Abstract
Purpose. It has been reported that intrathecal coadministration of a $GABA_A$ agonist and a glutamate antagonist induces synergistic antinociceptive effects in rats. We hypothesized that this synergistic antinociceptive effect might induce a synergistic reduction in the minimum alveolar anesthetic concentration (MAC).
Methods. The MAC for sevoflurane was determined before and after intrathecal administration of muscimol ($GABA_A$ agonist, 0.1–10 μg), AP-5 (*N*-methyl-D-aspartate [NMDA] antagonist, 0.1–10 μg), and YM872 (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid [AMPA] antagonist, 0.1–10 μg) in rats. The effects of coadministration of muscimol and AP-5 or YM872 on MAC reduction were also tested.
Results. Intrathecal administration of muscimol at doses of 1 and 10 μg significantly reduced the MAC by 34.5% ± 3.3% and 46.9% ± 4.4%, respectively ($P < 0.01$). Intrathecal administration of AP-5 at a dose of 10 μg and YM872 at a dose of 10 μg significantly reduced the MAC by 25.8% ± 2.4% and 31.4% ± 6.3%, respectively ($P < 0.01$). No additional reductions in MAC values were observed when a $GABA_A$ agonist was combined with either an NMDA or an AMPA antagonist.
Conclusion. Intrathecal coadministration of a $GABA_A$ agonist and an NMDA or AMPA antagonist, which has been reported to produce a synergistic antinociceptive effect, did not induce a synergistic reduction in the MAC. The antinociceptive effect may not be the predominant factor in the reduction of the MAC induced by these drugs.

Key words MAC · $GABA_A$ agonist · Glutamate antagonist

Introduction

Surgical immobility, which is one of the most important factors in general anesthesia, can be achieved by attenuation of nociception or depression of motor neuron excitability, and volatile anesthetics can produce immobility to noxious stimuli without the use of a muscle relaxant. It is not known, however, which factor is predominant in the production of immobility by volatile anesthetics.

There is a general consensus that spinal mechanisms play an important role in induced immobility [1–4]. GABA and glutamate are major neurotransmitters in spinal cord inhibitory and excitatory circuits, and it has been reported that volatile anesthetics can act on both receptors [5–8]. Furthermore, it has been shown that intrathecal administration of a $GABA_A$ receptor agonist or a glutamate receptor antagonist reduces the minimum alveolar anesthetic concentration (MAC; the anesthetic concentration that prevents movement in response to supramaximal stimulation in 50% of animals) of volatile anesthetics [9–11]. Therefore it is hypothesized that one or both of these receptors may be involved in the mechanism by which volatile anesthetics produce immobility.

It is well known that these receptors play an important role in spinal nociception. Increased efficacy of the GABA receptor can attenuate nociception by enhancing inhibition, and it has been reported that intrathecal administration of a GABA agonist has an antinociceptive effect in rats [12]. It has also been reported that intrathecal administration of a glutamate receptor antagonist has an antinociceptive effect in rats [13]. Considering these facts, it seems reasonable that the antinociceptive effect of intrathecal administration of a $GABA_A$ receptor agonist or a glutamate receptor antagonist may contribute to the reduction in MAC.

Recently, Nishiyama et al. [13] reported that intrathecal coadministration of midazolam, which enhances GABAergic activity, and a glutamate receptor antagonist (*N*-methyl-D-aspartate [NMDA] antagonist or α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid

Address correspondence to: H. Sumikura
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[AMPA] antagonist) synergistically prolonged tail-flick latency in rats. If attenuation of nociception plays an important role in the reduction in MAC after intrathecal administration of a $GABA_A$ receptor agonist or a glutamate receptor antagonist, we hypothesized that intrathecal coadministration of these combinations might reduce the MAC by synergistic actions. To test this hypothesis, two experiments were performed: the MAC was determined before and after intrathecal administration of a $GABA_A$ agonist, an NMDA antagonist, or an AMPA antagonist alone at various doses; and the MAC was determined before and after coadministration of a $GABA_A$ agonist plus an NMDA antagonist or a $GABA_A$ agonist plus an AMPA antagonist to determine whether the combinations acted synergistically.

Materials and methods

Animal preparations

The protocol was approved by the local animal care and use committee. Under pentobarbital anesthesia, male Sprague-Dawley rats weighing 300–350 g were implanted with intrathecal catheters according to the method described by Yaksh and Rudy [14]. After fixation of the head in a stereotaxic device, the intrathecal space was punctured through the atlantooccipital membrane, and a polyethylene catheter (PE10) was inserted and advanced to the lumbar enlargement and fixed by suture to the nuchal skin. After catheterization, the rats were allowed to recover for at least 5 days, and only rats with normal motor function and behavior were used. Measurement of the MAC was carried out 5 to 10 days after surgery. A total of 80 rats (45 administered only one drug, 30 coadministered two drugs, and 5 controls) were used.

Baseline MAC determination

The baseline MAC of each rat was determined to control for interindividual variation in the MAC. The rats were first anesthetized in a small anesthesia box with 5% sevoflurane in oxygen. After tracheostomy, the trachea was intubated with a 14-G intravenous catheter customized for continuous gas sampling with a small dead space. The lungs were ventilated thereafter with a tidal volume of 5 ml at a rate of 60 breaths·min^{-1} using an animal respirator. Throughout the experiments, the oxygen supply to the sevoflurane vaporizer was kept at 2 l·min^{-1}. The end-tidal concentrations of sevoflurane and CO_2 were monitored (sampling rate, 200 ml·min^{-1}) by an anesthetic gas monitor (M1025B, Hewlett Packard) [15]. The end-tidal CO_2 level was maintained between 35 and 45 mmHg by adjusting the respiratory rate. The rectal temperature was monitored and kept between 36.5° and 37.5°C throughout the experiment by a heating pad. The MAC of sevoflurane was determined according to the up-and-down method by the tail-clamp technique [16]. The determination was performed starting with an end-tidal concentration of 2.5%. Initially, 15 min was allowed for equilibration. A pair of 18-cm hemostat forceps was clamped to the first ratchet lock on the tail for 60 s. Gross movements of the head, extremities, or body were taken as positive signs, whereas grimacing, swallowing, chewing, and tail flicking were considered negative. According to the response, the sevoflurane concentration was increased or decreased by 0.2%, and 10 min was allowed for equilibration before the next stimulus. The first stimulus was applied to the proximal 2 cm of the end of the tail. The tail was always stimulated proximally to the previous test site. This procedure was repeated until changes in the response characteristics were obtained, and then the sevoflurane concentration was increased or decreased by 0.1% to study the response at the midpoint. The MAC was determined as the mean of the lowest concentration at which a negative response was observed and the highest concentration at which a positive response was observed.

Drugs and administration

Muscimol (5-aminomethyl-3-hydroxyisoxazole, a $GABA_A$ receptor agonist; Sigma, Deisenhofen Germany), 0.1, 1, and 10 μg, and AP-5 (2-amino-5-phosphonovaleic acid, an NMDA receptor antagonist; Sigma), 0.1, 1, and 10 μg, were dissolved in 10 μl of saline. YM872 ([2,3-dioxo-7-(1H-imidazol-1-yl)-6-nitro-1,2,3,4-tetrahydro-1-quinoxalinyl] acetic acid, an AMPA receptor antagonist; Yamanouchi Pharmaceutical, Tsukuba, Japan), 10 mg, was dissolved in 0.97 ml of distilled water with 30 μl of 1 N NaOH to adjust the pH to 7.3–7.5 [17]. Solutions of 0.1, 1, and 10 μg per 10 μl were made by using normal saline. After baseline determination of the MAC, the drugs were administered intrathecally, and the catheter was flushed with a subsequent injection of 10 μl of normal saline to clear the dead space of the catheter. Microinjector syringes were used for all injections. Fifteen rats were used for each drug. They were divided into three groups receiving three different doses ($n = 5$). Three syringes with different doses of the same drug were prepared, and the size of the dose was covered with color tape to keep it unknown to the test person. In the coadministration groups, the combinations of muscimol plus YM872 and muscimol plus AP-5 were tested at 0.1 μg + 0.1 μg, 1 μg + 1 μg, and 10 μg + 10 μg per 10 μl of saline. In the control group, the MAC was determined in 5 rats before and after intrathecal administration of saline.

Determination of the MAC after intrathecal administration of the drugs

Immediately after intrathecal administration of the drugs, the end-tidal sevoflurane concentration was adjusted to the highest concentration at which the rats had a positive response. After 15 min of equilibration, determination of the MAC was restarted. For the proceeding stimulations, 10 min was allowed for equilibration. According to the response, the sevoflurane concentration was increased or decreased by 0.2%, and 10 min was allowed for equilibration before the next stimulus. Because the effect of an intrathecally administered drug is not consistent over time and does not last for long, care was taken to reduce the pharmacokinetic effects of the intrathecally administered drugs. Determination of the MAC by using a dose-response curve to minimize the effect would have been better, but was not done because it would have required a large number of rats [18].

In the present study using the up-and-down method, an attempt to reduce the pharmacokinetic effects was made by standardizing the procedure and shortening the duration of MAC determination. To shorten the duration, 10 min was chosen for equilibration of sevoflurane, with the expectation that the small blood/gas partition coefficient of sevoflurane as compared with isoflurane might be possible, although 12–15 min has been used for equilibration to determine the MAC for isoflurane in previous studies [10,19]. To standardize the procedure, MAC determination was always restarted from the highest concentration at which the rats had a positive response, and no concentration was skipped to shorten the duration for determination of the MAC.

Statistical methods

All data are expressed as means ± SEM. The individual MAC reduction ratio was calculated as follows: %MAC reduction = (baseline MAC − post MAC)/baseline MAC.

The results were assessed statistically by an analysis of variance (ANOVA). The intergroup differences were analyzed by the Newman-Keuls test; $P < 0.05$ was considered to indicate statistical significance.

Results

The baseline MAC for sevoflurane obtained from all rats ($n = 80$) was 2.43% ± 0.21%, which is consistent with values previously reported for rats [20]. There were no significant differences among all groups in baseline MAC values. In the control group, there was no significant difference between the baseline MAC (2.41 ± 0.30) and the MAC after intrathecal administration of saline (2.37 ± 0.30).

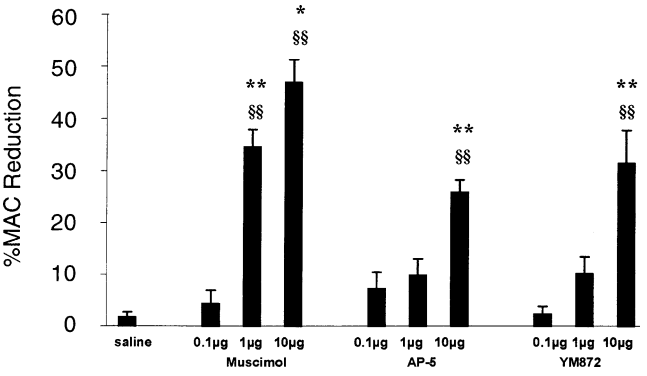

Fig. 1. Intrathecal administration of muscimol, AP-5, and YM872 decreases the minimum alveolar anesthetic concentration (MAC) values for sevoflurane. Data are presented as means ± SEM. §§$P < 0.01$ versus saline group, *$P < 0.05$ versus preceding concentration, **$P < 0.01$ versus preceding concentration

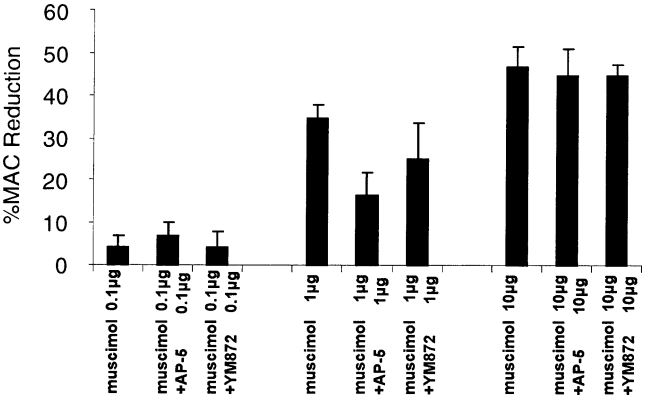

Fig. 2. Intrathecal coadministration of AP-5 and YM872 with muscimol does not decrease the MAC compared with muscimol alone. Data are presented as means ± SEM

Intrathecal administration of muscimol at doses of 1 and 10 µg significantly reduced the MAC by 34.5% ± 3.3% and 46.9% ± 4.4%, respectively (ANOVA and Newman-Keuls test, $P < 0.01$). Intrathecal administration of AP-5 at a dose of 10 µg and YM872 at a dose of 10 µg significantly reduced the MAC by 25.8% ± 2.4% and 31.4% ± 6.3%, respectively (ANOVA and Newman-Keuls test, $P < 0.01$) (Fig. 1). There were no significant differences in the MAC values after administration of muscimol alone and coadministration of muscimol plus AP-5 or muscimol plus YM872 (Fig. 2).

Discussion

The present study showed no additional reduction in the MAC value from the combination of a $GABA_A$

agonist with either an NMDA antagonist or an AMPA antagonist.

Reduction in the MAC after intrathecal administration of muscimol

The $GABA_A$ receptor has been considered a main target site of volatile anesthetics [5,6], and it has been reported that intrathecal administration of midazolam, which enhances GABA activity by activation of a GABA–benzodiazepine ionophore complex, reduce the MAC for isoflurane in rats [9]. However, to our knowledge, there are no reports of reduction in MAC by intrathecal administration of a specific $GABA_A$ agonist.

Muscimol is a specific $GABA_A$ agonist, and intrathecally administered muscimol possesses an antinociceptive effect and causes flaccidity. Although we did not perform a locomotion test in the present study, it has been demonstrated that 0.1 μg of muscimol, which prolongs tail-flick latency by more than 90 min, does not produce flaccidity; 1 μg of muscimol produces partial flaccidity for 60 min; and 10 μg of muscimol produces complete flaccidity for more than 180 min [12]. Therefore, it is suggested that lower doses of muscimol, which have an antinociceptive effect without producing flaccidity, do not reduce the MAC, whereas larger doses of muscimol, which have an antinociceptive effect and cause flaccidity, reduce the MAC significantly.

An attempt to determine the MAC after intrathecal administration of drugs at doses causing flaccidity may be criticized on the grounds that under total flaccidity rats cannot respond to noxious stimuli, regardless of sevoflurane concentration. It appears to be reasonable to claim that the significant reduction in the MAC at higher doses was biased by flaccidity, and lower doses of muscimol still decreased the MAC to some extent. It should be noted, however, that MAC determination was accomplished within 120 min for all rats in the group receiving 10 μg of muscimol, although it has been reported that 10 μg of muscimol produces complete flaccidity for more than 180 min [12]. In the group receiving 10 μg of muscimol, four of five rats showed a positive response by withdrawing their upper extremities or twisting their necks when their lower extremities did not show any response, and one rat showed a positive response by withdrawing both the upper and lower extremities at the same time. The former observation seems to imply that the motor response in the upper part of body was not completely inhibited, and the latter seems to imply that total flaccidity does not mean complete motor block. It is difficult to judge from the present result which of the effects of GABA, attenuation of nociception or depression of motor neurons, contributes the more to surgical immobility induced by volatile anesthetics. The present result, however, indicates that a dose of $GABA_A$ agonist sufficient to produce flaccidity cannot block nociception from the tail completely.

Recently, Zhang et al. [21] reported that intrathecal administration of a $GABA_A$ antagonist increased the MAC for isoflurane, but a ceiling effect was observed. Furthermore, the present study demonstrated that increasing the dose of $GABA_A$ agonist could not reduce the MAC to zero. A $GABA_A$ receptor might be involved in the production of immobility by volatile anesthetics, but the action of anesthetics on the $GABA_A$ receptor might not be the only mechanism by which volatile anesthetics produce immobility.

Reduction in the MAC after intrathecal administration of glutamate receptor antagonists

Volatile anesthetics have also been reported to attenuate excitatory neurotransmission in the central nervous system, particularly at glutamate synapses [7,8]. Intrathecal administration of a glutamate receptor antagonist has been reported to produce an antinociceptive effect [13,17] and reduce the MAC [10,22]. Therefore, it seems possible that volatile anesthetics reduce the MAC by this antinociceptive effect. To test this hypothesis, the MAC was determined before and after intrathecal administration of glutamate receptor antagonists at various doses. The excitatory effect of glutamate on nociception is mediated by at least two distinct classes of receptors, AMPA receptors and NMDA receptors. AMPA receptors are involved in mediating acute excitation from both A and C fibers, whereas NMDA receptors are involved in the facilitation of pain by repetitive stimulation of C fibers. In the present study, AP-5 was used as an NMDA antagonist, and YM-872, which is a potent and water-soluble competitive AMPA receptor antagonist originally developed as a neuroprotective drug for brain ischemia [17], was used as an AMPA antagonist.

To determine whether the antinociceptive effect contributes to MAC reduction, it was important to determine MAC while the antinociceptive effect remained. Therefore, efforts to shorten the duration for MAC determination were made, as described in the "Materials and Methods" section. As a result, MAC was determined within 90 min for most of the rats in these groups, although it has been reported that intrathecal administration of AP-5 (1 μg) and YM872 (0.3 μg) prolongs tail-flick latency to more than 90 min [23].

Ishizaki et al. [10] reported that intrathecal administration of AP-5 reduced the MAC, and our results are in agreement with theirs. Intrathecal administration of AP-5 at a dose of 1 μg, which has been reported to produce an antinociceptive effect without motor distur-

bance [13], did not reduce the MAC, and 10 μg, which has been reported to cause motor disturbance [13], reduced the MAC significantly.

The effect of AMPA antagonists on the MAC has been controversial. McFarlane et al. [22] reported a reduction in the MAC after systemic administration of an AMPA receptor antagonist, NBQX, which is a highly specific competitive AMPA antagonists. However, Ishizaki et al. [24] failed to show any reduction in the MAC after intrathecal administration of CNQX, which is an AMPA/kainate receptor antagonist. In the present study, intrathecal administration of YM872 at a dose of 1 μg, which has been reported to produce an antinociceptive effect without motor disturbance [17], did not reduce the MAC, and administration of 10 μg, which has been reported to cause flaccidity in 20% of rats [17], did reduce the MAC significantly. Hence, it is possible that the dose of CNQX used by Ishizaki et al. [24] did not induce flaccidity, whereas the dose of NBQX used by McFarlane et al. [22] did induce flaccidity.

The present study found a reduction in the MAC after intrathecal administration of glutamate antagonists. However, the reduction in the MAC by a small dose, which produces an antinociceptive effect but does not produce motor disturbance, was not significant. As discussed in the previous section, these significant differences may have been biased by flaccidity, and an antinociceptive dose may still reduce the MAC to some extent. However, it is apparent that intrathecal administration of glutamate agonist at doses that cause antinociception without flaccidity does not reduce the MAC profoundly. Recently, Cheng et al. [25] reported that enflurane exerts direct depressant effects on both NMDA and AMPA glutamate currents in motor neurons. Attenuation of nociception mediated by glutamate receptors may not contribute importantly reduction of the MAC by glutamate receptor antagonists.

Reduction in the MAC after coadministration of $GABA_A$ agonist and glutamate antagonist

Recently, it has been reported that intrathecal coadministration of midazolam and NMDA or AMPA receptor antagonists induces a synergistic antinociceptive effect [13]. We hypothesized that this synergistic antinociceptive effect might induce a synergistic reduction in the MAC, if attenuation of nociception is playing an important role in MAC reduction. However, we failed to show any further reduction in the MAC by adding NMDA or AMPA receptor antagonists to muscimol. The results from the group receiving small doses seem to be especially important, since they were not affected by the flaccidity. The present study does not show how much the attenuation of nociception contributes to the reduction in the MAC. However, our results suggest that the contribution of the attenuation of nociception to reduction in the MAC, if any, is limited.

The tail-flick and hot-plate tests have been used to elucidate the mechanism of spinal nociception after intrathecal coadministration of two drugs. Furthermore, reduction in the MAC after intrathecal administration of drugs has been studied to elucidate the mechanism of inhaled anesthetics in the spinal cord. Studying the reduction in the MAC after intrathecal coadministration of two drugs may elucidate the action of inhaled anesthetics on the excitability of the spinal cord.

In conclusion, intrathecal administration of a $GABA_A$ agonist or a glutamate receptor antagonist alone at a dose that caused motor disturbance significantly reduced the MAC. The MAC was not significantly reduced by low doses that have been reported to possess antinociceptive effects but do not produce motor disturbance. Coadministration of these drugs, which has been shown to produce synergistic antinociception, failed to produce a further reduction in the MAC. Since Rampil et al. [20] have demonstrated that inhaled anesthetics depress spinal motor neurons at clinical concentrations and suggested that anesthetic-induced immobility might be due to reduced excitability of motor neurons, attenuation of nociception mediated via GABA receptors, glutamate receptors, or both in the spinal cord may not be the predominant factor in the production of immobility by volatile anesthetics.

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