MK-801 enhances gabaculine-induced loss of the righting reflex in mice, but not immobility

[Le MK-801 accentue la perte du réflexe de redressement provoqué par la gabaculine chez les souris, mais pas l'immobilité]

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Purpose: γ-Aminobutyric acid (GABA) and N-methyl-D-aspartate (NMDA) receptors are important targets for anesthetic action at the *in vitro* cellular level. Gabaculine is a GABA-transaminase inhibitor that increases endogenous GABA in the brain, and enhances GABA activity. We have recently shown that unconsciousness is associated with the enhanced GABA activity due to gabaculine, but that immobility is not. MK-801 is a selective NMDA channel blocker. In this study, we examined behaviourally whether gabaculine in combination with MK-801 could produce these components of the general anesthetic state. We further compared the effect of MK-801 with ketamine, another NMDA channel blocker.

Methods: All drugs were administered intraperitoneally to adult male ddY mice. To assess the general anesthetic components, two endpoints were used. One was loss of the righting reflex (LORR; as a measure of unconsciousness) and the other was loss of movement in response to tail-clamp stimulation (as a measure of immobility).

Results: Large doses of MK-801 alone (10–50 $mg \cdot kg^{-1}$) induced neither LORR nor immobility in response to noxious stimulation. However, even a small dose (0.2 $mg \cdot kg^{-1}$) significantly enhanced gabaculine-induced LORR ($P < 0.05$), although gabaculine in combination with MK-801 (0.2–10 $mg \cdot kg^{-1}$) produced no immobility. However, gabaculine plus a subanesthetic dose of ketamine (30 $mg \cdot kg^{-1}$), which acts on NMDA, opioid and nicotinic acetylcholine receptors and neuronal Na^+ channels, suppressed the pain response, but did not achieve a full effect. Ketamine alone dose-dependently produced both LORR and immobility.

Conclusion: These findings suggest that gabaculine-induced LORR is modulated by blocking NMDA receptors, but that immobility is not mediated through GABA or NMDA receptors.

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***Objectif** : Les récepteurs GABA (acide gamma-aminobutyrique) et NMDA (N-méthyl-D-aspartate) constituent d'importantes cibles pour l'action des anesthésiques au niveau cellulaire in vitro. La gabaculine est un inhibiteur des GABA-transaminases qui augmente le GABA endogène dans le cerveau, et stimule l'activité GABA. Nous avons récemment démontré que la perte de conscience est associée à l'activité GABA stimulée par la gabaculine, mais que l'immobilité ne l'est pas. Le MK-801 est un bloqueur sélectif du canal NMDA. Dans cette étude, nous avons examiné si la gabaculine combinée à du MK-801 pouvait produire ces composantes de l'état d'anesthésie générale au niveau comportemental. Nous avons également comparé l'effet du MK-801 à celui de la kétamine, un autre bloqueur du canal NMDA.*

***Méthode** : Tous les médicaments ont été administrés à des souris mâles adultes ddY par voie intrapéritonéale. Deux paramètres ont été utilisés afin d'évaluer les composantes de l'anesthésie générale. L'un était la perte du réflexe de redressement (LORR – loss of righting reflex ; pour mesurer la perte de conscience), et l'autre l'absence de mouvement en réaction à la stimulation d'une pince à la queue (pour mesurer l'immobilité).*

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Résultats *: D'importantes doses de MK-801 seul (10-50 mg·kg^{-1}) n'ont provoqué ni LORR ni l'immobilité en réaction à une stimulation nociceptive. Toutefois, une dose même faible (0.2 mg·kg^{-1}) a significativement accentué le LORR provoqué par la gabaculine ($P < 0,05$), bien que la gabaculine associée à du MK-801 (0,2–10 mg·kg^{-1}) n'ait pas provoqué d'immobilité. Cependant, la gabaculine additionnée d'une dose sous-anesthésique de kétamine (30 mg·kg^{-1}), laquelle agit sur les récepteurs NMDA, opiacés et cholinergiques nicotiniques ainsi que sur les canaux Na^+, a supprimé la réaction douloureuse, mais n'a pas eu un effet complet. La kétamine seule a provoqué LORR et immobilité, de façon dose-dépendante.*

Conclusion *: Ces résultats suggèrent que le LORR provoqué par la gabaculine est modulé en bloquant les récepteurs NMDA, mais que l'immobilité n'est pas médiée par les récepteurs GABA ou NMDA.*

LIGAND-GATED ion channels are important targets for anesthetic action at the *in vitro* cellular level.[1,2] Chloride channels gated by the inhibitory neurotransmitter, γ-aminobutyric acid (GABA) are sensitive to clinical concentrations of a wide variety of general anesthetics, including halogenated inhalational agents and many intravenous (*iv*) agents.[3] At clinical concentrations, general anesthetics increase the sensitivity of the $GABA_A$ receptor to GABA, thus enhancing inhibitory neurotransmission and depressing nervous system activity. Ketamine, nitrous oxide and xenon have been shown to inhibit a different type of ligand-gated ion channel, the N-methyl-D-aspartate (NMDA) receptor, which is an excitatory amino acid receptor subtype. Ketamine,[4] nitrous oxide[5,6] and xenon[7,8] are potent inhibitors of NMDA-activated currents.

The general anesthetic state comprises behavioural and perceptual components, including amnesia, sedation, unconsciousness or hypnosis, analgesia, immobility in response to noxious stimulation and attenuation of autonomic responses to noxious stimulation.[1,9] The sedative and analgesic properties of nitrous oxide, a gaseous general anesthetic agent, may be modulated at different, independent sites.[10] Hence, it is imperative that each component be explored separately.[11] To assess the components of the general anesthetic state in the present study two endpoints were used. One was loss of the righting reflex (LORR; as a measure of unconsciousness) and the other was loss of movement in response to tail-clamp stimulation (as a measure of immobility).

The action of synaptically released GABA is terminated by uptake into neurons and glial cells. Released GABA is degraded by mitochondrial GABA-transaminase (GABA-T). Gabaculine is a potent, specific GABA-T inhibitor. The inhibitor increases endogenous GABA in the mammalian central nervous system. Subsequently, the increased GABA levels in the brain selectively enhance GABA activity.[12,13] We have recently shown in behavioural and microdialysis studies that endogenous GABA-induced LORR occurs in a brain concentration-dependent manner. However, even larger doses of gabaculine induce no immobility in response to noxious stimulation.[14] In contrast, intrathecal infusion of picrotoxin, a noncompetitive $GABA_A$ receptor antagonist, in rats increased the minimum alveolar concentration (MAC) required to suppress movement in response to noxious stimulation in 50% of subjects at approximately 40% with the inhaled anesthetics isoflurane, cyclopropane, and xenon.[15] MK-801 is a potent, selective NMDA channel blocker and easily passes through the blood-brain barrier.[16] It has been reported that intraperitoneal administration of small doses of MK-801 (0.3–3.0 mg·kg^{-1}) in mice increases the duration of LORR induced by the intravenous anesthetic pentobarbital, and reduces the MAC of the inhalational anesthetic halothane in a dose-dependent manner.[17] In contrast, neither intrathecal nor intravenous infusion of MK-801 in rats produced immobility in response to tail-clamp stimulation, although MK-801 decreases the MAC of isoflurane in a lower spinal cord concentration-dependent manner.[18] These findings indicate that the role of $GABA_A$ and NMDA receptors in inducing LORR and immobility remains a controversial issue. Different behavioural changes between drugs may occur because of the higher selectivity of MK-801 for the NMDA receptor channel compared to ketamine. Indeed, the affinity of MK-801 is over 1000-fold higher for the NMDA receptor channel than any of the sites with which ketamine interacts.[16]

To elucidate the functional relevance of enhancing GABA activity and of blocking NMDA receptors in mediating LORR and immobility in response to noxious stimulation, we examined behaviourally the effects of gabaculine in combination with MK-801 on the anesthetic components. In addition, the effect of MK-801 was compared with that of the intravenous anesthetic ketamine, which has been shown to act not only at NMDA receptors, but also at $GABA_A$, opioid and nicotinic acetylcholine receptors and in neuronal Na^+ channels.[19–21]

Methods

The studies were approved by the Committee of Research Facilities for Laboratory Animal Science, Hiroshima University School of Medicine.

Animals

Adult male ddY mice (Japan SLC Inc., Shizuoka, Japan) weighing 33–56 g were used in this study. Animals were housed five per cage in an air-conditioned room maintained at 25 ± 1°C with 50% relative humidity on a 12-hr light/dark cycle (lights on at 8:00 A.M.). Food and water were available *ad libitum*. Animals were used only once in all experiments. All behavioural experiments were performed between 10:00 A.M. and 6:00 P.M.

Drugs

3-Amino-2,3-dihydrobenzoic acid (gabaculine) hydrochloride and (5R,5S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine ([+]-MK-801) hydrogen maleate were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Ketamine hydrochloride was from Sankyo Co., Ltd. (Tokyo, Japan).

Gabaculine, MK-801, and ketamine were dissolved in 0.9% saline solution. Each drug was freshly prepared on the day of the experiment. All drugs were administered intraperitoneally in a volume of 0.05 mL per 10 g of body weight.

Measurement of general anesthetic potency

The general anesthetic state was evaluated using two endpoints: loss of rolling response (as a measure of unconsciousness) and loss of response to noxious stimulation (as a measure of immobility). In this study, the righting reflex was used to indicate the rolling response and clamping the mouse's tail was the noxious stimulation.

The mice were examined individually in a circular glass beaker (13.5 cm diameter × 19 cm high). To examine the righting reflex, we tilted the beaker by hand to an angle of approximately 45° from the horizontal plane. The beaker was tilted three times at each recording time after intraperitoneal administration of gabaculine, MK-801, ketamine, or vehicle saline. The righting reflex was assessed and recorded every two minutes for three hours after administration of each drug or saline by a blinded observer. Righting reflex scores were evaluated according to the rating scale of Irifune *et al.*:[22] a score of 0 indicated a normal righting reflex; +1 indicated that the mouse righted itself within two seconds on all three trials (slightly impaired righting reflex); +2 indicated that the latency to righting was > two seconds, but < ten seconds at the best response in the three trials (moderately or severely impaired righting reflex); +3 corresponded to absence of righting reflex (no righting within ten seconds on all three trials). The combination effect of MK-801, ketamine, or saline with gabaculine or saline was examined while the peak effect of gabaculine was occurring on the righting reflex. For this reason, MK-801 (0.2 $mg \cdot kg^{-1}$), ketamine (30 $mg \cdot kg^{-1}$), or saline was administered intraperitoneally 17 hr after the administration of gabaculine when the righting reflex scores peaked.[14]

To determine immobility, a tail clamp was applied with arterial forceps close to the base of the tail for one minute or until the animal moved at the time when each drug produced a peak effect on the righting reflex. The behavioural peak induced by each drug was consistent with peak brain or free plasma concentration.[14,23,24] Purposeful movement of head and/or legs after tail-clamp stimulation was considered a response. Purposeless movement, such as coughing or hyperventilation, was excluded.

Five to eight mice were used per dose for each drug, and five to six doses were used per dose response curve. The anesthetized animals were kept warm with an overhead heat lamp.

Statistical analysis

Because the response was all-or-none, the number of animals losing the righting reflex (scored +3) or the tail-clamp response of the total that received a specific treatment was used to calculate the percentage loss of response. The doses against percent effect of animals that lost the righting reflex or tail-clamp response were plotted on a logarithmic-probability scale graph. A straight line was fitted through the points, particularly those in the region of 40 to 60% effect. The line fitting was studied using the χ^2 test. The 50% effective dose (ED_{50}) for the LORR (righting-reflex ED_{50}) and for loss of movement in response to tail-clamp stimulation (tail-clamp ED_{50}) with 95% confidence limits, the parallelism between the dose-response curves and the significance of differences in ED_{50} values between groups were determined according to the method of Litchfield and Wilcoxon.[25] The ratio of tail-clamp ED_{50} to righting-reflex ED_{50} was calculated for each drug. The results were considered statistically significant when $P < 0.05$.

Results

The administration of gabaculine (35–200 $mg \cdot kg^{-1}$, *ip*) in combination with saline in the control group induced LORR in a dose-dependent fashion with an ED_{50} value of 100 (76–132; 95% confidence limits) $mg \cdot kg^{-1}$. Gabaculine at 200 $mg \cdot kg^{-1}$ [a 95% effective dose (ED_{95}) for LORR] induced LORR in all animals tested. However, even a large dose (400 $mg \cdot kg^{-1}$) produced no loss of movement in response to tail-clamp

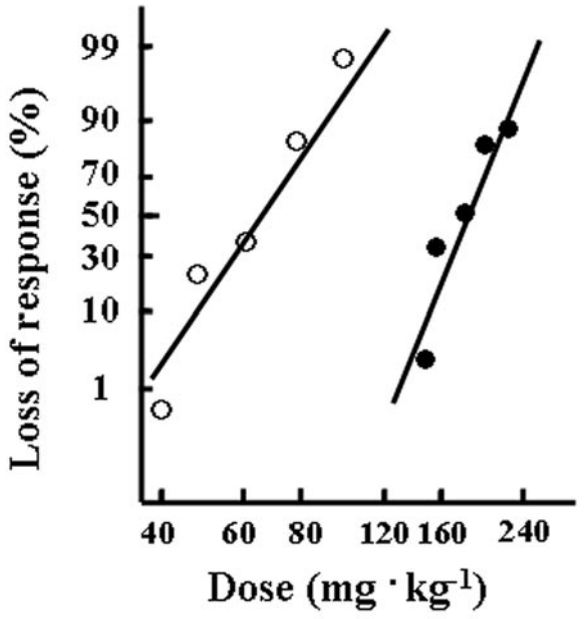

FIGURE 1 Effects of ketamine on the righting reflex (open circles) and tail-clamp response (closed circles) in mice. Ketamine was administered intraperitoneally. The righting reflex was assessed every two minutes for three hours after administration, and the tail-clamp response was evaluated when the drug produced its peak effect on the righting reflex (five to eight animals per dose, five doses per dose-response curve). The behavioural peak time induced by ketamine was consistent with the time when the agent's free plasma concentration peaked after systemic administration. The doses against percent effect of animals that lost the righting reflex or tail-clamp response are plotted on a logarithmic-probability (X-Y axis) scale graph. Each point represents the percent effect of five to eight animals per dose of an anesthetic agent (see Methods in the text for ED_{50} values).

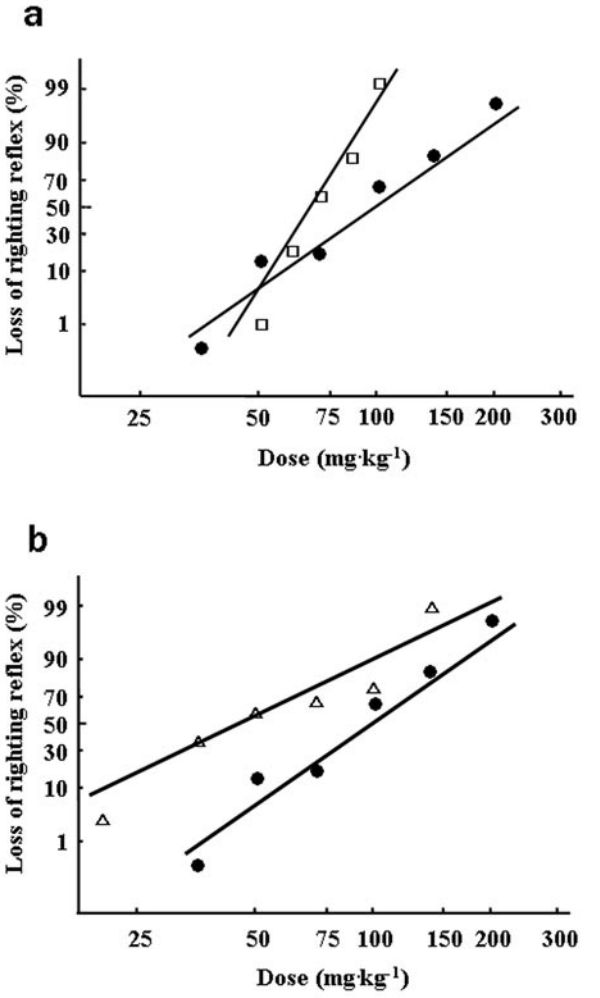

FIGURE 2A and B Effects of MK-801 (A) or ketamine (B) on gabaculine-induced loss of the righting reflex in mice. MK-801 (0.2 $mg \cdot kg^{-1}$; open squares), ketamine (30 $mg \cdot kg^{-1}$; open triangles) or vehicle (closed circles) was administered intraperitoneally 17 hr after the intraperitoneal injection of gabaculine. The doses of gabaculine were 50, 60, 70, 84, and 100 $mg \cdot kg^{-1}$ in the MK-801 group, 20, 35, 50, 70, 100, and 140 $mg \cdot kg^{-1}$ in the ketamine group, or 35, 50, 70, 100, 140, and 200 $mg \cdot kg^{-1}$ in the vehicle control group, respectively. The righting reflex was assessed every two minutes for three hours after the administration of each drug or vehicle (five to eight animals per dose, five to six doses per dose-response curve). The doses against percent effect of animals that lost the righting reflex are plotted on a logarithmic-probability (X-Y axis) scale graph. Each point represents a percent effect of five to eight animals per dose of gabaculine (see Methods in the text for ED_{50} values).

stimulation (Table). These results were consistent with our recent data.[14]

MK-801 alone (0.2–50 $mg \cdot kg^{-1}$, *ip*) induced neither LORR nor immobility in response to tail-clamping. Even a large dose (50 $mg \cdot kg^{-1}$) only slightly impaired the righting reflex (a score of +1), to an extent similar to the impairment caused by a dose of 1 $mg \cdot kg^{-1}$. The peak effect of MK-801 on the righting reflex scores occurred at approximately 30 min after the injection and the impaired righting reflex continued for more than two hours. In contrast, ketamine alone induced LORR in a dose-dependent manner (Figure 1). Because the peak effect of ketamine on the righting reflex occurred at six minutes post-injection, the tail-clamp stimulation was applied at the peak.

Ketamine prevented the tail-clamp response in a dose-dependent manner (Figure 1).

Larger doses of MK-801 (0.2–10 mg·kg^{-1}) and a small, subanesthetic dose of ketamine (30 mg·kg^{-1}) only slightly impaired the righting reflex (a score of +1). The combination of the NMDA channel blockers with gabaculine was examined while the peak effect of gabaculine was occurring on the righting reflex. For this reason, MK-801 (0.2 mg·kg^{-1}) and ketamine (30 mg·kg^{-1}) were administered intraperitoneally 17 hr after the administration of gabaculine when the righting reflex scores peaked. Both NMDA channel blockers enhanced gabaculine-induced LORR. MK-801 at 0.2 mg·kg^{-1} shifted the gabaculine dose-response curve for LORR to the left in a non-parallel manner (Figure 2a). The righting-reflex ED_{50} for gabaculine decreased significantly from 100 (76–132) mg·kg^{-1} to 68 (59–79) mg·kg^{-1} in the presence of MK-801 (0.2 mg·kg^{-1}) ($P < 0.05$). Ketamine at 30 mg·kg^{-1} also shifted the curve in a parallel manner (Figure 2b). The righting-reflex ED_{50} for gabaculine decreased significantly to 45 (32–64) mg·kg^{-1} in the presence of ketamine (30 mg·kg^{-1}) ($P < 0.05$) (Table).

The peak effect of MK-801 and ketamine on the righting reflex occurred at 30 min and six minutes post-injection, respectively; the tail-clamp stimulation was applied at the peak. A fixed dose of gabaculine (200 mg·kg^{-1}; ED_{95} for LORR) in combination with larger doses of MK-801 (0.2–10 mg·kg^{-1}) did not prevent the tail-clamp response (Table). In contrast, gabaculine in combination with ketamine (30 mg·kg^{-1}) dose-dependently reduced movement in response to the noxious stimulation, but did not achieve a full effect. The tail-clamp ED_{25} (a 25% effective dose) was 400 mg·kg^{-1} (Figure 3 and Table).

The Table summarizes the righting-reflex ED_{50}, the tail-clamp ED_{50} (ED_{25}) and the ratios of the tail-clamp ED_{50} (ED_{25}) to the righting-reflex ED_{50} for gabaculine alone, gabaculine in combination with NMDA channel blockers, and an intravenous anesthetic. In the gabaculine plus ketamine (30 mg·kg^{-1}) group, the ratio of tail-clamp ED_{50} (ED_{25}) to righting-reflex ED_{50} was approximately three times higher than that of ketamine alone.

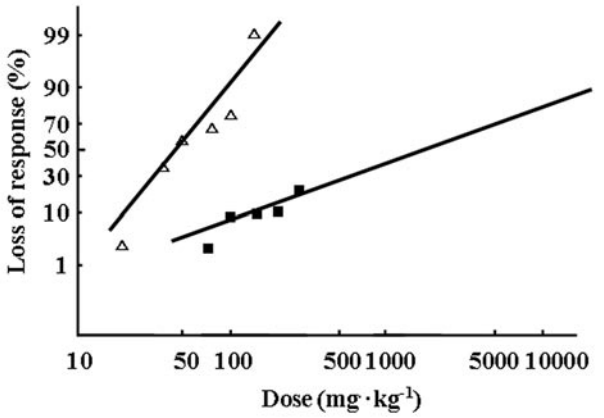

FIGURE 3 Effects of gabaculine in combination with ketamine on the righting reflex (open triangles) and tail-clamp response (closed squares) in mice. Ketamine (30 mg·kg^{-1}) was administered intraperitoneally 17 hr after the intraperitoneal injection of gabaculine. The doses of gabaculine were 20, 35, 50, 70, 100, and 140 mg·kg^{-1} for the righting reflex or 70, 100, 140, 200, and 285 mg·kg^{-1} for the tail-clamp response, respectively. The righting reflex was assessed every two minutes for three hours after the administration of ketamine, and the tail-clamp response was evaluated when the drug produced its peak effect on the righting reflex (five to eight animals per dose, five to six doses per dose-response curve). The doses against percent effect of animals that lost the righting reflex or tail-clamp response are plotted on a logarithmic-probability (X-Y axis) scale graph. Each point represents a percent effect of five to eight animals per dose of gabaculine (see Methods in the text for ED_{50} values).

Discussion

We showed in this study that a small dose of the specific NMDA channel blocker MK-801 enhanced gabaculine-induced LORR (as a measure of unconsciousness). However, gabaculine in combination with large doses of MK-801 produced no loss of movement in response to noxious stimulation (as a measure of immobility). These findings suggest that gabaculine-induced LORR is modulated by NMDA receptors, but that immobility is not associated with GABA or NMDA receptors.

Even large doses of MK-801 alone induced no LORR. However, ketamine, another NMDA channel blocker, dose-dependently produced LORR (Figure 1). In contrast to MK-801 alone, a small dose of MK-801 (0.2 mg·kg^{-1}) enhanced gabaculine-induced LORR and shifted the dose-response curve to the left in a non-parallel manner (Figure 2a). A small, subanesthetic dose of ketamine (30 mg·kg^{-1}) also shifted the gabaculine dose-response curve for LORR to the left in a parallel manner (Figure 2b). MK-801 appears to have a larger effect at increased doses of gabaculine on LORR than ketamine, which has a larger effect at the lower dose range of gabaculine. Thus, these differences between MK-801 and ketamine on the slope of the dose-response curves for LORR suggest a different mechanism of effect for these two drugs.

TABLE General anesthetic potency of a GABA mimetic, NMDA channel blocker, the combination of both, and an intravenous anesthetic

	Righting reflex ED_{50} ($mg \cdot kg^{-1}$)	*Tail-clamp ED_{50} (ED_{25}) ($mg \cdot kg^{-1}$)*	*Tail-clamp ED_{50} (ED_{25})/ Righting-reflex ED_{50}*
GABA mimetic + NMDA channel blocker			
Gabaculine			
+ saline	100 (76–132)	ND	ND
+ MK-801 (0.2 $mg \cdot kg^{-1}$)	68 (59–79)*	ND	ND
+ MK-801 (10 $mg \cdot kg^{-1}$)	NT	ND	ND
+ ketamine (30 $mg \cdot kg^{-1}$)	45 (32–64)*	(400)	(8.89)
Saline			
+ saline	ND	ND	ND
+ MK-801 (0.2 $mg \cdot kg^{-1}$)	ND	ND	ND
+ ketamine (30 $mg \cdot kg^{-1}$)	ND	ND	ND
Intravenous anesthetic			
Ketamine	66 (56–77)	180 (164–198)	2.73

Values are ED_{50} (ED_{25}) (95% confidence limits) for loss of righting reflex (righting-reflex ED_{50}), for loss of tail-clamp response [tail-clamp ED_{50} (ED_{25})] and ratio of tail-clamp ED_{50} (ED_{25}) to righting-reflex ED_{50} [tail-clamp ED_{50} (ED_{25})/righting-reflex ED_{50}]. *$P < 0.05$ compared to gabaculine alone. GABA = γ-Aminobutyric acid; NMDA = N-methyl-D-aspartate; ED = effective dose; ND = not detectable; NT = not tested.

Gabaculine at doses four times larger than the ED_{50} for LORR induced no immobility in response to tail-clamp stimulation. Larger doses of MK-801 (50–100 $mg \cdot kg^{-1}$) alone also produced no loss of tail-clamp response. Furthermore, gabaculine in combination with large doses of MK-801 (0.2–10 $mg \cdot kg^{-1}$) elicited no immobilization. These findings suggest that the enhanced GABA activity and the selective blockade of NMDA channels are related to LORR, but not to immobility.

It has been demonstrated that immobility in response to a surgical incision (the endpoint used in determining MAC) results from inhaled anesthetic action in the spinal cord.[26–28] Gabaculine was administered systemically in the present study. Therefore, it is problematic to interpret the effects of systemically administered drugs mechanistically, unless effective concentrations of the drugs can be found at the targets. Systemic administration of gabaculine increases GABA concentrations in both the intracellular and extracellular compartments of the rat brain,[29] suggesting the penetration of gabaculine through the blood-brain barrier. However, no reports have shown that systemic gabaculine increases GABA levels in the spinal cord.

The blood-cerebrospinal fluid barrier and the blood-brain barrier exist between the blood and cerebrospinal fluid and brain fluid, respectively. Low permeability of the blood-cerebrospinal fluid and blood-brain barriers occurs in the same manner in which the endothelial cells of the capillaries are joined to one another. They are joined by so-called tight junctions. The cerebrospinal fluid is found in the ventricles of the brain, in the cisterns around the brain, and in the subarachnoid space around both the brain and spinal cord. All these chambers are connected with one another.[30] Therefore, gabaculine should also be present in the spinal cord because the drug penetrates the blood-brain barrier and increases brain GABA after systemic injection.[14,29] Moreover, gabaculine has been shown to block specifically and irreversibly GABA-T in dissociated cell cultures from the spinal cord. The inactivation of the enzyme leads to an increase in GABA levels.[31] To elucidate the mechanism of penetration of gabaculine through the blood-cerebrospinal fluid barrier, however, further studies will be required.

Gabaculine in combination with large doses of MK-801 (0.2–10 $mg \cdot kg^{-1}$) induced no immobility, suggesting that immobility is not mediated through GABA or NMDA receptors. MK-801 has been shown to pass through the blood-cerebrospinal fluid barrier. After continuous intravenous infusion of MK-801 (50 $\mu g \cdot kg^{-1} \cdot min^{-1}$) for 228 ± 47 (mean ± SD) min, the concentration of MK-801 in the lower spinal cord was 6.76 ± 1.57 $\mu g \cdot g^{-1}$ tissue.[18] In the present study, we did not determine the concentration of MK-801 in the spinal cord after intraperitoneal administration. However, the dose of MK-801 (10 $mg \cdot kg^{-1}$, *ip*) used may have been sufficient to induce behavioural changes via neuronal pathways in the spinal cord because it has been reported that a small dose of MK-801 (0.05 $mg \cdot kg^{-1}$, *ip*) inhibits the nociceptive response caused by intrathecal injection of NMDA in mice.[32]

Contrary to the present findings, intrathecal administration of MK-801 decreased isoflurane MAC in a lower spinal cord concentration-dependent manner.[18] Volatile anesthetics, including isoflurane, work on a

variety of molecular targets, consistent with their status as complete anesthetics, affecting all components.[1] Thus, the selective blockade of NMDA channels by MK-801 may stimulate some of the molecular targets involved in inducing immobility.

It is of interest that gabaculine in combination with a small fixed dose of ketamine (30 $mg \cdot kg^{-1}$) dose-dependently induced immobility, but only partially (Figure 3 and Table). Ketamine has been shown to act not only at NMDA receptors, but also at $GABA_A$, opioid and nicotinic acetylcholine receptors and in neuronal Na^+ channels.[19–21] Thus, these effects of ketamine on the receptors and channels may be involved in the production of immobility. The partial effect of gabaculine in combination with ketamine on immobility may be due to the small dose of ketamine. In fact, a large dose of ketamine alone achieved a full effect (Figure 1).

The ratio of the tail-clamp ED_{25} to the righting-reflex ED_{50} of gabaculine plus a small dose of ketamine (30 $mg \cdot kg^{-1}$) was approximately three times higher than those of the tail-clamp ED_{50} to the righting-reflex ED_{50} of ketamine alone (Table), suggesting that this treatment-induced behaviour occurs via mechanisms different from the one induced by ketamine alone. The dose of 30 $mg \cdot kg^{-1}$ of ketamine is much smaller than the 5% effective dose (ED_5) of the drug alone for immobility, that is, 150 $mg \cdot kg^{-1}$. In fact, this dose of ketamine alone induced no immobility. Thus, gabaculine may potentiate those effects of ketamine which are associated with inducing immobility.

It has been reported that β3 subunit-containing $GABA_A$ receptors do not mediate immobility produced by inhaled anesthetics.[33] In addition, conventional anesthetics do not produce immobility in response to noxious stimulation by blocking the action of glutamate on NMDA receptors.[34] These reports support the present findings that gabaculine, MK-801, and the combination of both do not induce immobility.

In conclusion, our findings suggest that gabaculine-induced LORR is modulated by blocking NMDA receptors, but that immobility is not mediated through GABA or NMDA receptors.

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