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Intravenous Anesthetics Inhibit Nonadrenergic Noncholinergic Lower Esophageal Sphincter Relaxation via Nitric Oxide–Cyclic Guanosine Monophosphate Pathway Modulation in Rabbits

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Background: **Nonadrenergic noncholinergic (NANC) nerves have important roles in the regulation of the lower esophageal sphincter (LES) motility and function. The effects of thiopental, ketamine, and midazolam on NANC LES relaxation were investigated.**

Methods: **The isometric tension of circular muscle strips from Japanese White rabbits was examined. The NANC relaxation was induced by KCl (30 mM) in the presence of atropine (3×10^{-6} M) and guanethidine (3×10^{-6} M). The modifications of the NANC and sodium nitroprusside (SNP; 10^{-5} M)-induced relaxation by the anesthetics were examined. The content of 3',5'-cyclic guanosine monophosphate (cGMP) was measured by radioimmunoassay.**

Results: **The KCl-induced relaxation was abolished by pretreating with tetrodotoxin (10^{-6} M). The NANC relaxation was inhibited in the presence of N^G-nitro-L-arginine (L-NNA; 3×10^{-5} M), methylene blue (10^{-6} M), apamin (10^{-7} M), and glibenclamide (10^{-5} M). The SNP-induced relaxation was inhibited by methylene blue but was not affected by tetrodotoxin, L-NNA, apamin, or glibenclamide. Ketamine ($EC_{50} = 8.8 \times 10^{-5}$ M) and midazolam ($EC_{50} = 4.8 \times 10^{-6}$ M) suppressed the NANC response in a concentration-dependent manner, leaving SNP-induced response unchanged. Thiopental altered neither of the relaxations. cGMP content was decreased in the presence of ketamine and midazolam.**

Conclusion: **The NANC relaxation was mediated by nitric oxide and by low-conductance calcium- and adenosine triphosphate–sensitive potassium channels of smooth muscle. The modulation of the nitric oxide–cGMP pathway was related, at least in part, to the inhibitory actions of ketamine and midazolam on the NANC LES relaxation.**

VARIOUS neurotransmitters, hormones, and peptides released from extrinsic and intrinsic innervations contribute to the regulation of gastrointestinal motility and function. Nonadrenergic noncholinergic (NANC) nerves have important roles in mediating peristaltic waves, inhibitory responses, and/or relaxing mechanisms of the gastrointestinal tract, including the lower esophageal sphincter (LES),[1] which is a specialized smooth muscle situated at the esophagogastric junction. Nitric oxide (NO), NO-related substance, and/or some inhibitory neuropeptides have been demonstrated as neurotransmitters mediating NANC relaxation in the opossum,[2] canine,[3] and cat[4] LES. In recent years, NO has been considered to mediate physiologic responses of the esophagus and LES.[5] The LES relaxes with swallowing, and NO is considered to be associated with the swallowing-induced response of the esophageal body and LES.[6] In pathologic conditions, NO or NO-related substance is considered to be associated with the etiology of gastroesophageal reflux disease.[7]

In clinical anesthetic practice, many intravenous and volatile anesthetics have decreased LES pressure.[8] Previous investigations in intubated adult patients have revealed that deepening of anesthesia by halothane depresses lower esophageal contractility[9] and that a decrease in lower esophageal *p*H followed induction of anesthesia.[10] It has also been reported that a laryngeal mask airway insertion[11,12] or a cricoid cartilage compression[13] induces decreases in LES pressure. Recently, intravenous midazolam has been reported to produce abnormal esophageal motility in healthy volunteers.[14]

The mechanism of LES tone modifications or esophageal motility disorders induced by anesthetic agents has not been fully elucidated.[15] The interaction of the neurotransmitter NO mediating the NANC relaxation, between myenteric plexus and smooth muscle cell, and the course of the relaxation induced by sodium nitroprusside (SNP) are shown in figure 1. The current study was designed, first, to demonstrate the mechanism of NANC relaxation from rabbit LES strips. Second, as the neurotransmitters mediating NANC LES relaxation in other animals have been demonstrated as NO or NO-related substances,[2,3] this study was designed to test the hypothesis that the intravenous anesthetics thiopental, ketamine, and midazolam affect NANC transmission by the modulation of the NO–3',5'-cyclic guanosine monophosphate (cGMP) pathway, using isometric tension recording and the radioimmunoassay of cGMP.

Materials and Methods

The experimental protocol was approved by the Okayama University Animal Use Committee. Forty-one

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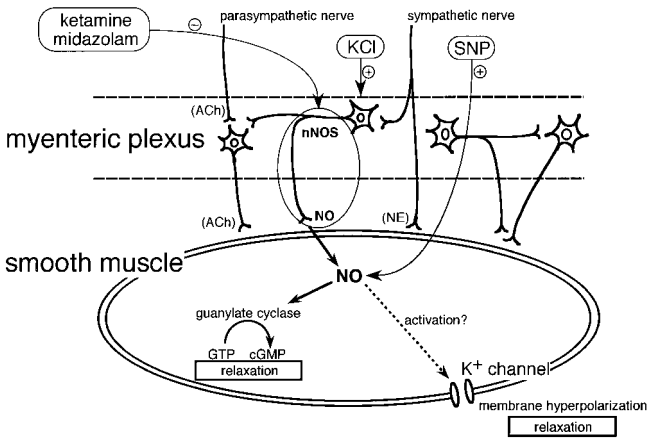

Fig. 1. The mechanism of smooth muscle relaxation regarding the relation between the myenteric plexus and smooth muscle cells, and the interaction of neurotransmitters mediating the nonadrenergic noncholinergic (NANC) and sodium nitroprusside (SNP)-induced relaxation of the lower esophageal sphincter are summarized. Nitric oxide (NO), mediating the NANC relaxation, is released from the myenteric plexus and delivered into smooth muscle cells, which results in the activation of guanylate cyclase and the accumulation of 3',5'-cyclic guanosine monophosphate (cGMP). The NANC relaxation could also be mediated by membrane hyperpolarization, which is induced by the activation of the K^+ channel of smooth muscle. The relation between intracellular NO and K^+ channel activation was not demonstrated in this study; however, K_{Ca} channel activation by NO has been reported in vascular smooth muscle. SNP, the exogenous NO donor, directly acts on smooth muscle cells and is metabolized to NO inside the smooth muscle cells. Ketamine and midazolam act on the process of NO production, including neuronal nitric oxide synthase (nNOS) activation in the myenteric plexus. ACh = acetylcholine; NE = norepinephrine; GTP = guanosine 5'-triphosphate.

adult male Japanese White rabbits weighing between 2 and 3 kg were anesthetized with thiopental sodium (50 mg/kg administered intravenously) and killed by exsanguination. The lower part of the esophagus and stomach were immediately isolated. The esophagogastric junction was opened along the longitudinal axis, and the LES was excised by sharp circular cutting, making strips approximately 2 mm wide and 5 mm long. The mucosa was removed.

The strips were vertically fixed between two hooks under a resting tension of 1.0 g, and the hook anchoring the upper end was connected to a force-displacement transducer. Changes in the isometric tension of circular muscle were recorded. The strips were suspended in a thermostatically controlled (37.0 ± 0.5°C) 20-ml organ bath containing Krebs-Ringer solution (118 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 1.18 mM KH_2PO_4, 1.19 mM $MgSO_4$, and 11 mM glucose). The bath fluid was aerated with a mixture of 95 % O_2 and 5 % CO_2 to keep the pH at 7.35–7.45. Before starting the experiments, the strips were allowed to equilibrate for 60 min in the Krebs solution, which was replaced every 15 min. In this series of experiments, drugs were applied directly to the organ bath by micropipette.

The effects of tetrodotoxin (10^{-6} M) and extracellular Ca^{2+} depletion on 30 mM KCl-induced relaxation were examined. To deplete extracellular Ca^{2+}, $CaCl_2$ in the Krebs-Ringer solution was replaced with 2 mM EGTA. Thereafter, NANC relaxation was induced by 30 mM KCl in the presence of atropine (3×10^{-6} M) and guanethidine (3×10^{-6} M). The modification of the NANC relaxation was examined in the absence or presence of N^G-nitro-L-arginine (L-NNA; 3×10^{-5} M) and methylene blue (10^{-6} M), which are nonspecific inhibitors of NO synthase (NOS) and the inhibitor of guanylate cyclase, respectively. The effects of apamin (10^{-7} M), charybdotoxin (10^{-7} M), and glibenclamide (10^{-5} M), which are inhibitors of the low-conductance calcium-sensitive potassium (K_{Ca}) channel, high-conductance K_{Ca} channel, and adenosine triphosphate–sensitive potassium (K_{ATP}) channel, respectively, on the NANC relaxation were also examined. Modification of the NANC response in the presence of [Ac-Tyr^1, D-Phe^2]-GRF 1-29 amide (vasoactive intestinal peptide [VIP] antagonist; 10^{-6} M) was also estimated. Maximal relaxation was confirmed by papaverine (10^{-4} M).

The nature of the relaxation induced by SNP (10^{-5} M), an exogenous NO donor, was examined. The modification of this response in the absence and presence of tetrodotoxin, L-NNA, methylene blue, apamin, charybdotoxin, and glibenclamide were examined, similar to the NANC relaxation.

The relaxation of LES was also induced by pinacidil (10^{-5} M), a potent opener of the K_{ATP} channel, and the effect of glibenclamide on this response was investigated.

The effects of thiopental, ketamine, and midazolam on the NANC relaxation and the SNP-induced relaxation were analyzed. Thiopental and ketamine were pretreated for 10 min at concentrations of 10^{-7} to 3×10^{-4} M for ketamine, and 10^{-7} to 10^{-3} M for thiopental. Midazolam was pretreated for 10 min at concentrations of 10^{-7} to 3×10^{-5} M.

As the available antagonists in this study, atropine, guanethidine, tetrodotoxin, L-NNA, methylene blue, and VIP antagonist were pretreated for 10 min. The K^+-channel blockers apamin, charybdotoxin, and glibenclamide were pretreated for 10 min.

For the radioimmunoassay of cGMP, ketamine (10^{-5} M, 10^{-4} M, 3×10^{-4} M) and midazolam (3×10^{-6} M, 10^{-5} M, 3×10^{-5} M) were applied to LES strips for 10 min in the presence of atropine and guanethidine, and the strips were frozen in liquid nitrogen 2 min after the application of KCl. In a preliminary study, cGMP content was measured under the condition that the time intervals were 10 s, 30 s, 1 min, 2 min, and 3 min between the application of KCl and freezing of muscle strips in liquid nitrogen. The maximal content of cGMP was obtained at time interval of 2 min. In the control study, an equivalent volume of distilled water was applied in the presence of

atropine and guanethidine using another strip. The strips were then homogenized in 6% volume-to-volume ratio trichloroacetic acid. The homogenate was centrifuged at 3,000 rpm for 10 min, the supernatant fractions were subjected to ether extraction and subsequent succinylation, and the pellet was analyzed for protein content. cGMP in each sample was radioimmunoassayed using a Yamasa assay kit (Yamasa Shoyu Co., Chiba, Japan). Levels of cGMP in tissues were expressed as picomoles per mg of protein. The effect of membrane-permeable cGMP analog, N^2,2'-*o*-dibutyrylguanosine 3',5'-cyclic monophosphate (dibutylyl cGMP) on resting LES tension was also examined.

The following drugs were used: methylene blue and potassium chloride from Nacalai Tesque, Kyoto, Japan; ketamine hydrochloride, atropine sulfate, guanethidine hydrochloride, glibenclamide, L-NNA, SNP, tetrodotoxin, apamin, charybdotoxin, and dibutylyl cGMP from Sigma Chemical, St. Louis, MO; midazolam from Yamanouchi Pharmaceutical Co., Tokyo, Japan; thiopental from Tanabe Pharmaceutical Co., Osaka, Japan; and VIP antagonist from Peninsula Laboratories, Belmont, CA. Glibenclamide and midazolam were dissolved in 1 N HCl and diluted with distilled water into 10 times the initial concentration. The final concentration of HCl in the bath was less than 3×10^{-4} N. In a preliminary study, 3×10^{-4} N HCl did not induce any effect on isometric tension of the muscle. Other drugs were dissolved in distilled water and handled in siliconized glassware.

Statistical Analysis

The results were expressed as mean values ± SD. One-way analysis of variance was used to examine the differences in the effects among the doses of anesthetic agents, and a Bonferroni test was used as a *post hoc* comparison to test for the statistical significance between control values *versus* drug-treated values. The Mann–Whitney U test was used to test the significance of the difference between the NANC and SNP-induced response groups. For all statistical tests, a P value < 0.05 was regarded as significant.

Results

The application of 30 mM KCl induced a transient relaxation and was followed by a sustained strong contraction that may have been caused by muscle membrane depolarization (fig. 2). The KCl-induced relaxation was abolished by pretreating with tetrodotoxin (fig. 3A), a nonspecific neural toxin, and by the depletion of extracellular Ca^{2+} (fig. 3B), suggesting that this relaxation is mediated by neural activity. The relaxation induced by 30 mM KCl was observed in the presence of atropine and guanethidine (fig. 3C, a). SNP (10^{-5} M) also induced LES relaxation, which mimicked the NANC response with respect to the amplitude of relaxation and the increase time to the maximal relaxation (fig. 3C, b).

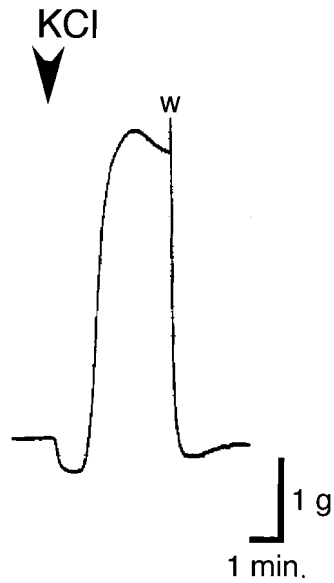

Fig. 2. Effects of 30 mM KCl on strips from the lower esophageal sphincter. KCl induced a transient relaxation followed by sustained contraction induced by membrane depolarization. The arrow indicates the application of KCl.

The NANC response was significantly reduced in the presence of L-NNA ($P < 0.01$), methylene blue ($P < 0.01$), apamin ($P < 0.05$), and glibenclamide ($P < 0.01$) (table 1). However, the application of VIP antagonist or charybdotoxin did not affect the NANC relaxation. The SNP-induced relaxation was significantly reduced by methylene blue ($P < 0.01$) but was not affected by tetrodotoxin (fig. 3D), L-NNA, apamin, charybdotoxin, or glibenclamide (table 1). The application of tetrodotoxin, L-NNA, methylene blue, and glibenclamide did not affect the resting tension; however, apamin and charybdotoxin induced slight contraction of LES strips (baseline elevated 0.2–0.3 g).

The application of pinacidil, a K_{ATP} channel opener, induced a relatively long-term relaxation, and pretreatment with glibenclamide almost completely inhibited this response (fig. 4).

Ketamine and midazolam reduced the NANC relaxation, but not the SNP-induced relaxation, in a concentration-dependent manner, comparing each of the relaxations without anesthetic agents taken as 100% (figs. 5A and 5B). A significant difference was observed between the NANC and SNP-induced response groups, which were pretreated with ketamine and midazolam. The decrease in the NANC response by ketamine was significant at concentrations of 10^{-4} M ($P < 0.01$) and 3×10^{-4} M ($P < 0.01$). The decrease in the NANC response by midazolam was significant at concentrations of 10^{-5} M ($P < 0.01$) and 3×10^{-5} M ($P < 0.01$). The ED_{50} values for the inhibition of the NANC relaxation were 8.8×10^{-5} M for ketamine and 4.8×10^{-6} M for midazolam. Thiopental did not alter either of the

Table 1. Effects of Various Inhibitors on the Nonadrenergic Noncholinergic (NANC) Relaxation and the SNP-induced Relaxation of Isolated Rabbit LES Strips

	Control	L-NNA	Methylene Blue	Apamin	Charybdotoxin	Glibenclamide	VIP Antagonist
NANC relaxation	100	67 ± 10* (N = 10)	60 ± 13* (N = 7)	27 ± 24* (N = 6)	119 ± 12 (N = 6)	68 ± 16* (N = 12)	96 ± 8 (N = 5)
SNP-induced relaxation	100	117 ± 14 (N = 6)	76 ± 4* (N = 6)	105 ± 12 (N = 5)	107 ± 8 (N = 5)	75 ± 15 (N = 6)	NT

* Significantly different from each of the control values in the absence of agents.

SNP = sodium nitroprusside; LES = lower esophageal sphincter; L-NNA = N^G-nitro-L-arginine; VIP = vasoactive intestinal peptide; NT = not tested.

relaxations; no significant difference was observed between the NANC and SNP-induced response groups (fig. 5C). Because high concentrations of thiopental ($\geq 3 \times 10^{-4}$ M) induced a direct relaxing effect, the NANC and SNP-induced relaxation cannot be precisely estimated. Figure 6 shows a typical tension record displaying a decrease in the NANC response with increasing concentrations of ketamine.

The content of cGMP was decreased in the presence of ketamine (fig. 7A) and midazolam (fig. 7B). cGMP content was significantly decreased by ketamine at concentrations of 10^{-5} M ($P < 0.05$), 10^{-4} M ($P < 0.05$), and 3×10^{-4} M ($P < 0.01$), and by midazolam at concentrations of 3×10^{-6} M ($P < 0.01$), 10^{-5} M ($P < 0.01$), and 3×10^{-5} M ($P < 0.01$). Figure 8 shows the concentration–response relation of the membrane-permeable cGMP analog, dibutylyl cGMP, taking the NANC relaxation as 100%. The application of dibutylyl cGMP induced LES relaxation; the maximal relaxation obtained (10^{-4} M) was approximately 70–80 % of the NANC relaxation.

Discussion

The relaxation induced by KCl was abolished by pretreating with tetrodotoxin, suggesting some neurotransmitter released from myenteric plexus (Meissner plexus) or submucosal plexus (Auerbach plexus) mediates this response. The relaxation induced by KCl in the presence of atropine and guanethidine was considered to be a NANC response. One of the neurotransmitters mediating NANC LES relaxation could be NO, because this response was inhibited by L-NNA and methylene blue, which are inhibitors of NOS and guanylate cyclase. These findings are in accordance with previous investigations using other animal species.[2–4] With Ca^{2+}-free medium, the KCl-induced relaxation was also abolished. This may be a result of the suppression of neurotransmitter release at the endplate in the myenteric plexus. In previous studies, electrical field stimulation was used for production of NANC relaxation.[2–4] However, in rabbit LES, electrical field stimulation during various conditions

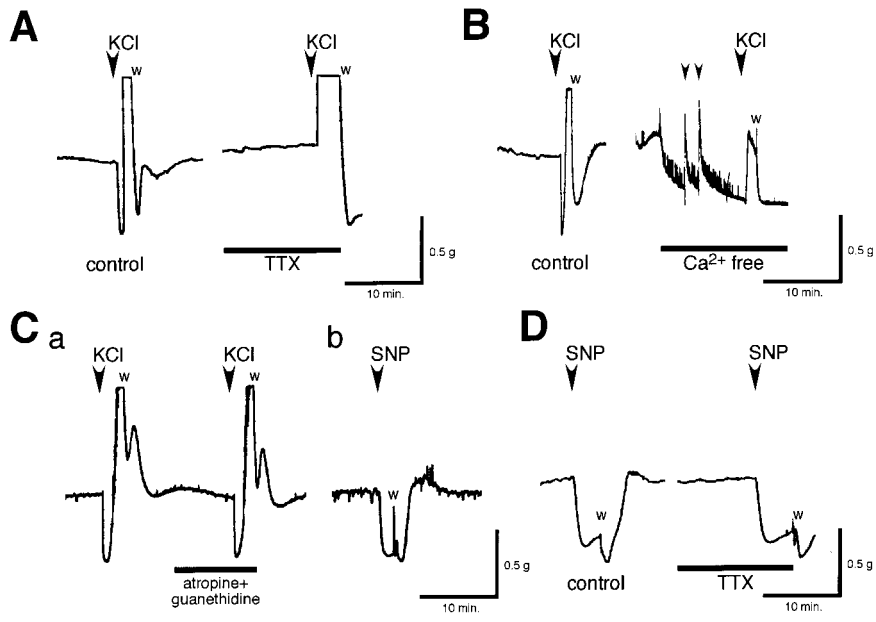

Fig. 3. (*A*) Effect of 10^{-6} M tetrodotoxin (TTX) on 30 mM KCl-induced relaxation of strips from the lower esophageal sphincter. KCl-induced relaxation was abolished by TTX. To magnify the relaxation, the component of KCl-induced contraction is overscaled (*A–C*). Arrows indicate the application of KCl. Horizontal bars indicate the presence of TTX. (*B*) Effects of extracellular Ca^{2+} depletion on 30 mM KCl-induced relaxation of strips from the lower esophageal sphincter. KCl-induced relaxation was abolished by the depletion of extracellular Ca^{2+}. Large arrows indicate the application of KCl. As the depletion of extracellular Ca^{2+} induced spontaneous decrease in resting tension, adjustment of the resting tension was performed (small arrows). Horizontal bar indicates the application of Ca^{2+}-depleted Krebs-Ringer solution. (*C*) Effects of 30 mM KCl in the absence and presence of atropine and guanethidine (*a*), and 10^{-5} M sodium nitroprusside (SNP) (*b*) on strips from the lower esophageal sphincter are shown. The relaxation induced by KCl in the presence of atropine and guanethidine is considered to be nonadrenergic noncholinergic response. The relaxation induced by KCl mimics that induced by SNP with regard to the amplitude of relaxation and to the increase time to the maximal relaxation. Arrows indicate the application of KCl and SNP. Horizontal bar indicates the presence of atropine and guanethidine. (*D*) Effect of 10^{-6} M TTX on 10^{-5} M SNP-induced relaxation of strips from the lower esophageal sphincter. SNP-induced relaxation was not affected by TTX. Arrows indicate the application of SNP. Horizontal bars indicate the presence of TTX.

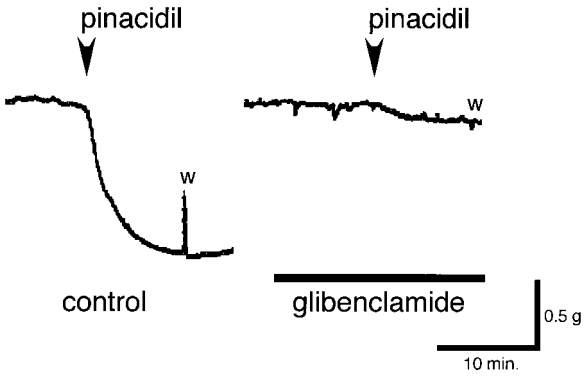

Fig. 4. Direct relaxing effect of 10^{-5} M pinacidil on a strip from the lower esophageal sphincter is shown. Pinacidil-induced relaxation was antagonized by pretreating with 10^{-5} M glibenclamide. Arrows indicate the application of pinacidil. Horizontal bar indicates the presence of glibenclamide.

never induced muscle relaxation but did induce contraction that is sensitive to tetrodotoxin.[16] Therefore, we used chemical stimulation by KCl to induce NANC response. VIP has been reported as one of the neurotransmitters mediating NANC LES relaxation.[17,18] However, pretreating with VIP antagonist did not affect the NANC response in rabbit.

In recent years, it has been suggested that membrane hyperpolarization is associated with relaxation of vascular smooth muscles.[19] The opening of the smooth muscle K^+ channel induces membrane hyperpolarization, and subsequent suppression of voltage-dependent Ca^{2+} channel decreases Ca^{2+} entry, which results in vasodilation.[19] In the current study, the NANC response was inhibited by apamin and glibenclamide. This finding suggested that low-conductance K_{Ca} and K_{ATP} channel activation are associated with this relaxation. Intracellular NO may activate K_{Ca} channels because the activation of the K_{Ca} channel by NO has been demonstrated in vascular smooth muscle.[20–22] The relaxation induced by pinacidil was inhibited by glibenclamide, suggesting that the K_{ATP} channel exists in rabbit LES smooth muscle. The prevalence of the K_{ATP} channel has been demonstrated in cardiac muscle, smooth muscle, skeletal muscle, and cerebral cortical neurons.[23]

The relaxation induced by SNP was reduced by methylene blue but was not affected by tetrodotoxin, L-NNA, apamin, charybdotoxin, or glibenclamide. Therefore, SNP could act directly on smooth muscle without affecting NOS activation or smooth muscle K^+ channel activation. It has been reported that SNP is metabolized into NO or compounds closely related to NO inside the smooth muscle cell and increases the intracellular cGMP content.[24]

The effects of intravenous anesthetics on LES tone and esophageal motility in humans appear to be still unclear; diazepam has been reported to decrease LES pressure in normal subjects,[25] midazolam to produce esophageal peristaltic dysfunction without affecting LES pressure in

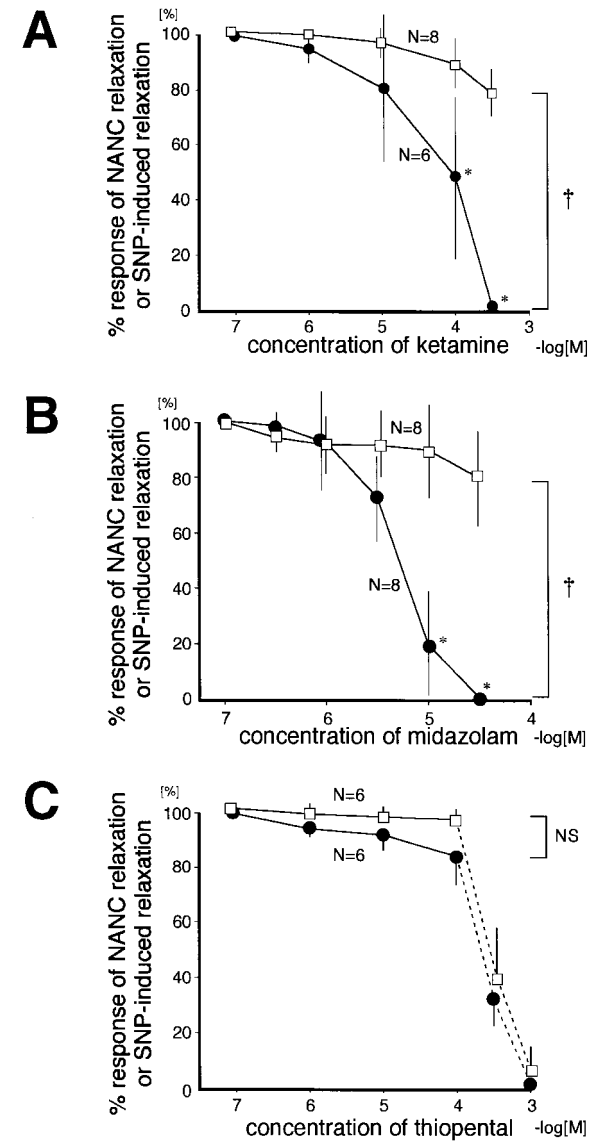

Fig. 5. The concentration–response relation of ketamine (*A*), midazolam (*B*), and thiopental (*C*) on 30 mM KCl-induced relaxation in the presence of atropine and guanethidine (nonadrenergic noncholinergic [NANC] relaxation; closed circle) or on 10^{-5} M sodium nitroprusside (SNP)-induced relaxation (open square) of strips from the lower esophageal sphincter are shown. Both ketamine and midazolam inhibited the NANC relaxation in a concentration-dependent manner, leaving the SNP-induced relaxation unchanged. Thiopental did not affect either of these two relaxations. The curve is expressed as a percent response of each relaxation in the absence of each anesthetic agent. Each point represents the mean from tissues from three to five animals; vertical lines show SDs. N = number of strips. *Significantly different from the value in the absence of ketamine, midazolam, or thiopental; †significantly different between the NANC and SNP-induced response groups; NS = not significant.

normal subjects,[14] and no clinical data are available for ketamine. The effects of anesthetics on the NANC LES relaxation, the exact site of anesthetic action, has not been elucidated in animals. In the current study, ketamine and midazolam suppressed the NANC relaxation in a concentration-dependent manner, which was medi-

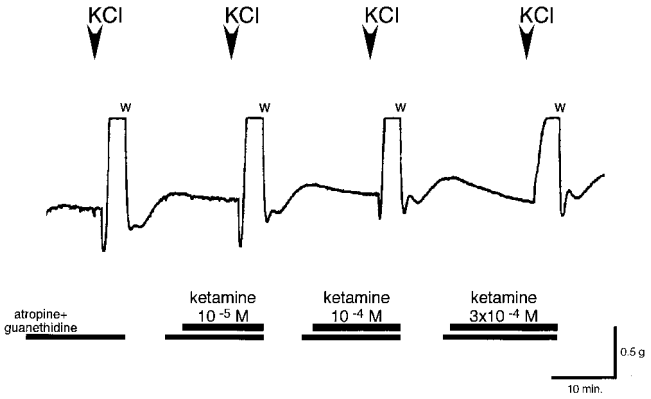

Fig. 6. The effect of increasing concentrations of ketamine on 30 mM KCl-induced relaxation in the presence of atropine and guanethidine (nonadrenergic noncholinergic relaxation) of a strip from the lower esophageal sphincter is shown. The nonadrenergic noncholinergic relaxation is inhibited in a concentration-dependent manner by pretreatment with ketamine. To magnify the relaxation, the component of KCl-induced contraction is overscaled. Arrows indicate the application of KCl. Upper horizontal bars indicate the presence of ketamine. Lower horizontal bars indicate the presence of atropine and guanethidine.

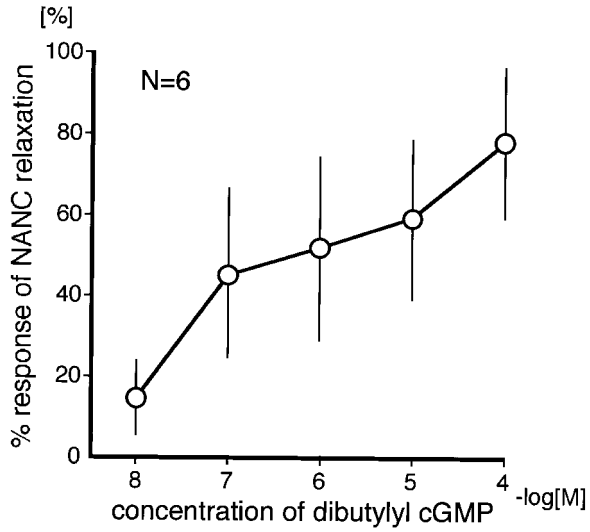

Fig. 8. The concentration–response relation of N^2,2'-*o*-dibutyryl-guanosine 3',5'-cyclic monophosphate (dibutylyl cGMP) on strips from the lower esophageal sphincter is shown. Dibutylyl cGMP induced a concentration-dependent lower esophageal sphincter relaxation. The curve is expressed as a percent response of 30 mM KCl-induced relaxation in the presence of atropine and guanethidine (nonadrenergic noncholinergic [NANC] relaxation) in the absence of dibutylyl cGMP. N = number of strips.

ated by the activation of NOS and/or smooth muscle K^+ channel, but not the SNP-induced relaxation, which was not mediated by the activation of NOS or K^+ channel. This suggests ketamine and midazolam could be involved in the production of NO or in the activation of the K^+ channel of the smooth muscle. The content of cGMP in smooth muscle after pretreatment with ketamine and midazolam was significantly decreased; therefore, it is likely that the site of action of these anesthetics could be the process of NO production, including NOS activation, in the myenteric plexus. However, the possibility that ketamine and midazolam directly inhibit the K^+ channel of the smooth muscle cannot be excluded. The possible site of action of the anesthetic agents is also indicated in figure 1. The suppression of NANC relaxation by ketamine and midazolam were observed almost within the clinical plasma concentration levels, which has been reported as less than 10^{-4} M for ketamine and as 10^{-6} to 10^{-5} M for midazolam,[26] with regard to their EC_{50} values obtained in the current study. Thiopental did not alter either of the two relaxations. Higher concentrations of thiopental, greater than 3×10^{-4} M, directly relaxed LES smooth muscle. This could be a result of the inhibition of transmembrane influx of Ca^{2+} *via* the inactivation of L-type Ca^{2+} channel. The suppression of the NANC and SNP-induced relaxation in high concentrations of thiopental may be a nonspecific response that is mediated by K_{Ca} channel inactivation induced by the decrease in intracellular Ca^{2+}.

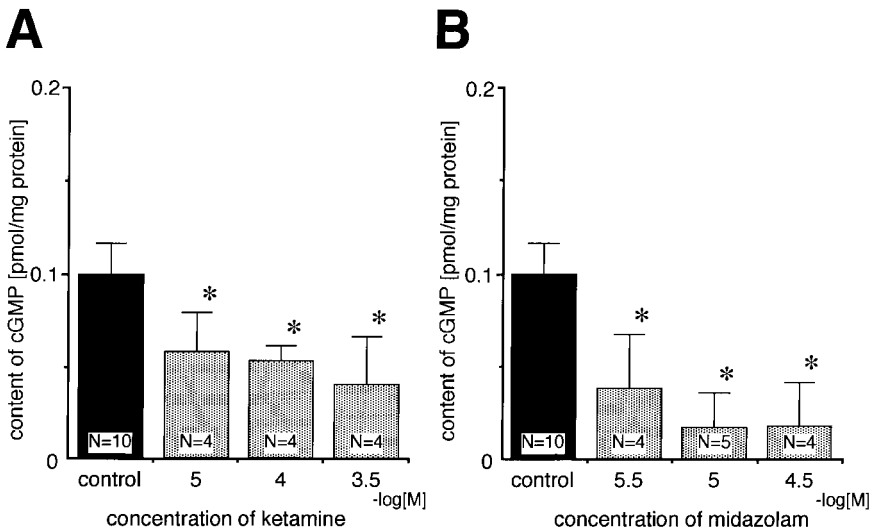

Fig. 7. Content of 3', 5'-cyclic guanosine monophosphate (cGMP) in strips from the lower esophageal sphincter in the absence (closed bars) or presence of 10^{-5}, 10^{-4} or 3×10^{-4} M ketamine (dotted bars) (*A*) and 3×10^{-6}, 10^{-5}, or 3×10^{-5} M midazolam (dotted bars) (*B*) 2 min after the application of 30 mM KCl in the presence of atropine and guanethidine are shown. For the control study, 30 mM KCl was replaced by distilled water in the presence of atropine and guanethidine. Each bar represents the mean from tissues from three to four animals; vertical lines show SDs. N = number of strips. *Significantly different from the value in the absence of anesthetic agents.

The content of cGMP in LES strips in our study appear to be small in comparison to investigations using vascular smooth muscle. However, previous studies using LES smooth muscle revealed the basal cGMP level to be almost similar to that in our study.[27,28] The application of the membrane-permeable cGMP analog relaxed LES strips to 70–80% of the NANC relaxation. The NANC relaxation and the inhibition of the NANC relaxation by ketamine and midazolam cannot be explained by the increase and decrease in intracellular cGMP alone, and other mechanisms, such as K^+ channel activation and/or membrane hyperpolarization, may be involved.

In the current study, we showed the indirect effects of ketamine and midazolam inhibiting NANC relaxation, the concentration of which did not affect resting tension. However, the direct relaxing effect of ketamine was not observed, except at higher concentration such as 3×10^{-4} M (fig. 6). We previously reported that ketamine relaxes LES smooth muscle directly, and the direct relaxing effect is a result of the activation of the adenylate cyclase–3',5'-cyclic adenosine monophosphate pathway and to the inhibition of transmembrane influx of Ca^{2+}.[15] The direct relaxing effect of ketamine is slow in onset and sustained, taking approximately 20–25 min to reach maximal relaxation. During the pretreatment period of 10 min in the current study, the resting tension did not show such decrease as the NANC relaxation could not be assessed even at 3×10^{-4} M. We did not examine the direct effect of midazolam on LES strips; however, the application of midazolam at a concentration of 3×10^{-5} M did not affect the resting tension.

Recently, interest in the interaction of anesthetic agents and NOS has increased. It has been reported that NOS inhibition dose-dependently decreased the anesthetic requirements of thiopental, propofol, and ketamine in *Xenopus laevis*[29] and decreased the minimum alveolar concentration for halothane in rats.[30] Thiopental, ketamine, and midazolam have been reported to inhibit NOS activity in the rat brain[31]; however, other studies reported that volatile anesthetics, not intravenous anesthetics, inhibit the activity in rat brain NOS.[26] These findings imply some interaction between brain NOS and anesthetic agents. However, the interactions between anesthetic agents and NOS that is distributed in the gastrointestinal tract has not been elucidated. In LES of various animal species, it has been demonstrated that neuronal NOS exists in the myenteric plexus or submucosal plexus.[32,33] In the current study, the possibility that ketamine and midazolam inhibited neuronal NOS activity cannot be excluded.

Studies using healthy adult volunteers revealed the effectiveness of NOS inhibitors on gastroesophageal reflux episodes by decreasing the frequency of transient LES relaxation, which is an abnormal long-lasting relaxation and has been demonstrated as the etiology of gastroesophageal reflux disease.[6] In the current study, we showed that ketamine and midazolam inhibited the NANC relaxation of rabbit LES, probably by inhibiting NO production. Therefore, these drugs may be expected to prevent LES pressure decrease during laryngeal mask airway insertion or cricoid cartilage compression, by inhibiting the maneuver-induced transient LES relaxation.[13]

In conclusion, the NANC relaxation of rabbit LES is mediated by NOS activation and neurotransmitter NO, which is released from the myenteric plexus or submucosal plexus. In smooth muscle, it is conducted *via* the low-conductance K_{Ca} and K_{ATP} channels. It is possible that ketamine and midazolam inhibit the process of NO production in the myenteric plexus or submucosal plexus. The modulation of the NO–cGMP pathway relates, at least in part, to the inhibitory actions of ketamine and midazolam on LES NANC transmission.

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