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Effects of GABAergic agents on anesthesia induced by halothane, isoflurane, and thiamylal in mice

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Abstract

The effects of γ-aminobutyric acid (GABA) receptor modulators and GABA uptake inhibitors on volatile and intravenous anesthetic-induced anesthesia were examined in male ICR mice, as assessed by the loss of righting reflex (LORR). The GABA uptake inhibitors, NO-711 and SKF89976A, which are permeable to the blood–brain barrier (BBB), but not nipecotic acid or guvacine, which poorly permeate BBB, shortened the onset of LORR but did not affect the duration of LORR induced by 1.5% halothane and 2% isoflurane. NO-711 and SKF89976A shortened the onset of and prolonged the duration of LORR induced by thiamylal (45 mg/kg ip). The GABA mimetics, muscimol and diazepam, shortened the onset of and prolonged the duration of LORR induced by halothane, isoflurane, and thiamylal. On the other hand, picrotoxin, a $GABA_A$ receptor antagonist, prolonged the onset of LORR induced by all anesthetics tested. Another $GABA_A$ receptor antagonist, bicuculline, prolonged the onset of LORR induced by halothane, but not by isoflurane or thiamylal. Both antagonists failed to affect the duration of LORR induced by halothane, isoflurane, or thiamylal. Baclofen, a $GABA_B$ receptor agonist, enhanced both volatile anesthetics- and thiamylal-induced anesthesia. These results suggest that anesthesia induced by volatile and intravenous anesthetics might be correlated with the modification of the pre- and/or postsynaptic GABAergic activities.

Keywords: GABA; General anesthetics; GABA uptake inhibitors; GABA mimetics; GABA receptor antagonists; Anesthesia; Righting reflex

1. Introduction

Although the mechanisms underlying anesthesia are not well elucidated and remain controversial, it appears likely that the brain's primary inhibitory neurotransmitter system such as γ-aminobutyric acid (GABA) is involved in anesthesia, since it has been shown that numerous classes of anesthetic and hypnotic agents including volatile anesthetics, barbiturates, and benzodiazepines enhance endogenous GABA-mediated inhibition of neuronal activity in the mammalian nervous system (see reviews Franks and Lieb, 1998; Little, 1996; Pocock and Richards, 1993; Tanelian et al., 1993). There is direct evidence indicating the interaction of anesthetics with $GABA_A$ receptor demonstrated by in vitro electrophysiological studies. Jones and Harrison (1993) showed that volatile anesthetics altered the gating kinetics of $GABA_A$ receptor to prolong the duration of GABA-mediated synaptic inhibition. Volatile anesthetics have been also found to reduce spontaneous firing of neocortical neurons in culture mainly by increasing the $GABA_A$ receptor-mediated mechanism (Antkowiak and Helfrich-Forster, 1998). However, in vivo elucidation of the relationship between the central GABAergic system and volatile or intravenous anesthetic-induced anesthesia have not been fully carried out. In vivo behavioral studies revealed that $GABA_A$ receptor agonists increase and the antagonists decrease barbiturate-induced hypnosis (Chweh et al., 1987; Sivam et al., 1982). There is also evidence indicating the interaction of the volatile anesthetic halothane and benzodiazepines in producing anesthesia in mice (Chambers et al., 1978; Geller et al., 1989; Moody and Skolnick, 1988). Recently, Irifune et al. (1999) reported that propofol anesthesia in mice was potentiated by muscimol and reversed by bicuculline. However, the mechanisms of

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the action of volatile anesthetics have yet to be extensively elucidated in vivo.

Facilitation of GABAergic inhibitory neuronal systems can be induced by several mechanisms different from the potentiation of postsynaptic $GABA_A$ receptor function. Potential presynaptic mechanisms include: decreased GABA metabolism, enhanced GABA release, and decreased GABA uptake, as suggested by in vitro studies using brain slices and synaptosomes (see review Griffiths and Norman, 1993). The purpose of the present study was to elucidate in vivo the involvement of pre- and postsynaptic GABAergic mechanisms in anesthesia using a behavioral model in mice. To this end, we examined the effects of GABA uptake inhibitors on anesthesia induced by the volatile anesthetics halothane and isoflurane together with the intravenous anesthetic barbiturate, thiamylal. These effects were compared with those of GABA receptor mimetics or antagonists.

2. Methods

2.1. Animals

The present study was approved by the Committee of Research Facilities for Laboratory Animal Science, Hiroshima University School of Medicine. We used 4–5-week-old ICR male mice (SLC, Japan), weighing 16–24 g.

2.2. Drugs

GABA uptake inhibitors, (±)-nipecotic acid (Sigma, USA), SKF89976A hydrochloride (Tocris, USA), guvacine hydrochloride (Tocris), NO-711 hydrochloride (Sigma), $GABA_A$ receptor agonist, muscimol (Sigma), $GABA_B$ receptor agonist, baclofen (Ciba-Geigy; Novartis, Japan), $GABA_A$ receptor antagonists, picrotoxin (Katayama Chemical, Japan), bicuculline (Sigma) were dissolved in 0.9% saline. The benzodiazepine receptor agonist, diazepam (Wako, Japan) was dissolved in 0.1 ml ethanol and added to 9.9 ml saline. Each drug solution was freshly prepared on the day of the experiment. All drugs described above were administered intraperitoneally (ip) at a volume of 10 ml/kg. Control animals received equal volume of vehicles. The doses and pretreatment times of these drugs were nipecotic acid: 200 and 400 mg/kg, 30 min; SKF89976A: 1 and 2 mg/kg, 30 min; guvacine: 10 and 20 mg/kg, 30 min; NO-711: 1 and 2 mg/kg, 30 min; muscimol: 0.5 and 1 mg/kg, 15 min; baclofen: 10 and 20 mg/kg, 10 min; diazepam: 1 and 2 mg/kg, 10 min; picrotoxin: 1 and 2 mg/kg, 5 min; and bicuculline: 1, 2, and 4 mg/kg, 10 min. A preliminary study confirmed that these agents at the doses used caused neither loss of righting reflex (LORR) nor convulsion by themselves.

Experiments with volatile anesthetics were conducted in a flow-through system (Chambers et al., 1978; Geller et al., 1989; Moody and Skolnick, 1988). Based on the preliminary examination of concentration (1% to 3%), exposure time (3 to 10 min), and flow rate (2 to 4 l/min) of volatile anesthetics, 1.5% halothane (Zeneca Takeda Chemical Industries, Japan), and 2% isoflurane (Abott, USA) were administered from the calibrated Fluotec 3 (for halothane) and Forwick (for isoflurane) vaporizer at a flow rate of 4 l/min oxygen for 5 min. The vaporizer was connected to a plastic box with internal dimensions of $21 \times 15 \times 8$ cm, and inlet and outlet holes of 1.0 cm diameter on opposite faces. Anesthetic concentrations in the chamber were monitored using an infrared analyzer. Intravenous anesthetic thiamylal (45 mg/kg ip) (Yoshitomi Pharmaceutical, Japan) was administered at a volume of 10 ml/kg.

2.3. Estimation of anesthesia

The anesthetic action was assessed using the righting reflex. After administration of anesthetics, the plastic box was inclined gradually and mice were placed on their backs at 15-s intervals. Animals were considered to have lost their righting reflex when they did not right themselves for more than 10 s. They were considered to have regained that ability when all four paws touched the ground simultaneously upon landing (Chambers et al., 1978; Geller et al., 1989; Moody and Skolnick, 1988).

After pretreatment with GABAergic agents or vehicles, animals were individually placed in the plastic box and exposed to 1.5% halothane or 2% isoflurane for 5 min. The onset of the LORR was defined as the time between the start of exposure to anesthetics and the loss. After 5 min exposure, mice were removed from the plastic box and placed on their back in an open cage. The duration of LORR was determined as the time between the removal from the box and the recovery of the righting reflex (Chambers et al., 1978; Geller et al., 1989; Moody and Skolnick, 1988). For thiamylal anesthesia, the onset of LORR was the time between the injection of thiamylal and the LORR. The duration of LORR in thiamylal anesthesia was the time between the onset of and the emergence from LORR.

2.4. Statistical analysis

The results were analyzed by a one-way factorial analysis of variance (ANOVA) with Fisher's PLSD for post hoc test. Statistical significance was assumed where P values were less than .05.

3. Results

3.1. Effects of GABAergic agents on the onset of LORR induced by halothane, isoflurane, and thiamylal

Effects of pre- and postsynaptic GABAergic agents on the onset of LORR induced by halothane (1.5%), isoflurane

(2%), and thiamylal (45 mg/kg ip) in mice are summarized in Table 1.

Both SKF89976A (2 mg/kg) and NO-711 (2 mg/kg), GABA uptake inhibitors known to be permeable to the blood–brain barrier (BBB) (Borden et al., 1994; Nielsen et al., 1991; Suzdak et al., 1992), but not nipecotic acid (up to 400 mg/kg) or guvacine (up to 20 mg/kg), which are less permeable to the BBB, significantly shortened the onset of LORR induced by halothane and isoflurane. Similar effects of these agents were observed in thiamylal-induced anesthesia.

All the GABA mimetics, muscimol (0.5 and/or 1 mg/kg), baclofen (10 and 20 mg/kg), and diazepam (1 and/or 2 mg/kg) shortened the onset of LORR induced by halothane, isoflurane, and thiamylal. On the other hand, picrotoxin (2 mg/kg), a $GABA_A$ receptor antagonist, prolonged the onset of LORR induced by halothane, isoflurane, and thiamylal. Another $GABA_A$ receptor antagonist, bicuculline at doses of 2 and 4 mg/kg shortened the onset of LORR induced by halothane, but failed to affect the onset of LORR induced by isoflurane or thiamylal.

3.2. Effects of GABAergic agents on the duration of LORR induced by halothane, isoflurane, and thiamylal

Table 2 shows the effects of GABAergic agents on the duration of LORR induced by halothane, isoflurane, and thiamylal.

All the GABA uptake inhibitors at the doses tested failed to change the duration of LORR induced by volatile anesthetics, except that NO-711 and SKF89976A at doses of 1 and/or 2 mg/kg prolonged the duration of LORR induced by halothane and isoflurane, but these differences were not significant. However, NO-711 (2 mg/kg) and SKF89976A (2 mg/kg), but not nipecotic acid (up to 400 mg/kg) or guvacine (up to 20 mg/kg), significantly prolonged sleeping time induced by thiamylal.

All the GABA mimetics examined profoundly prolonged the duration of LORR induced by halothane, isoflurane, and thiamylal (muscimol at 1 mg/kg for halothane and isoflurane and 0.5 and 1 mg/kg for thiamylal; baclofen at 20 mg/kg for halothane and isoflurane and 10 and 20 mg/kg for thiamylal; diazepam at 1 and 2 mg/kg for halothane and

Table 1
Effects of GABAergic agents on the onset of LORR induced by halothane, isoflurane, and thiamylal

		Onset of LORR (s)		
Drugs and doses (mg/kg)		Halothane	Isoflurane	Thiamylal
GABA uptake inhibitor				
Saline		74.9 ± 2.2 (11)	81.8 ± 1.9 (11)	102.8 ± 3.5 (8)
Nipecotic acid	200	74.3 ± 1.7 (8)	81.0 ± 4.1 (8)	95.0 ± 4.2 (8)
	400	73.8 ± 2.1 (8)	76.8 ± 4.3 (8)	91.3 ± 2.8 (8)
NO-711	1	70.2 ± 2.5 (10)	70.4 ± 1.9* (10)	87.0 ± 4.5* (9)
	2	62.6 ± 2.8* (10)	63.6 ± 3.2**	86.9 ± 2.5* (10)
Guvacine	10	71.2 ± 3.2 (10)	83.5 ± 5.0 (10)	103.2 ± 5.6 (9)
	20	76.0 ± 3.1 (10)	82.9 ± 2.2 (10)	104.1 ± 6.5 (10)
SKF89976A	1	70.7 ± 1.1 (10)	65.3± 2.1** (10)	85.0 ± 4.1* (10)
	2	68.0 ± 1.5* (10)	63.9 ± 2.0** (10)	82.5 ± 5.2* (10)
GABA mimetics				
Saline		82.2 ± 3.2 (10)	83.5 ± 3.7 (10)	104.1 ± 4.1 (11)
Muscimol	0.5	75.0 ± 2.3 (10)	59.7 ± 2.0** (10)	84.3 ± 6.6* (9)
	1	57.4 ± 6.3** (10)	37.5 ± 4.1** (8)	83.1 ± 8.4* (9)
Saline		84.1 ± 3.9 (11)	83.5 ± 3.7 (10)	113.5 ± 9.9 (10)
Baclofen	10	67.5 ± 2.1** (10)	60.0 ± 1.9** (8)	89.4 ± 4.3** (10)
	20	66.9 ± 3.0** (10)	47.5 ± 2.8** (10)	62.3 ± 4.9** (10)
Saline		82.4 ± 3.1 (11)	83.5 ± 3.7 (10)	131.8 ± 11.2 (11)
Diazepam	1	78.3 ± 2.6 (10)	67.3 ± 2.5* (9)	101.3 ± 8.7 (11)
	2	63.5 ± 3.1** (10)	52.5 ± 4.7** (8)	95.5 ± 8.9* (10)
GABA antagonist				
Saline		86.5 ± 4.1 (11)	76.1 ± 3.1 (9)	104.1 ± 4.1 (11)
Picrotoxin	1	91.2 ± 1.6 (10)	83.6 ± 4.7 (10)	108.6 ± 4.2 (8)
	2	107.5 ± 4.7** (10)	107.5 ± 2.1** (8)	125.0 ± 5.0** (8)
Saline		82.5 ± 2.9 (11)	76.1 ± 3.2 (9)	104.1 ± 4.1 (11)
Bicuculline	1	92.3 ± 4.2 (11)	84.7 ± 5.3 (10)	107.2 ± 5.1 (9)
	2	97.5 ± 4.1* (13)	73.5 ± 3.1 (8)	109.4 ± 11.5 (9)
	4	100.8 ± 5.6* (13)	80.0 ± 1.3 (8)	115.6 ± 12.2 (9)

Values are means ± S.E.M. Numbers in parentheses represent the number of animals.

* $P < .05$ vs. saline control.

** $P < .005$ vs. saline control.

Table 2
Effects of GABAergic agents on the duration of LORR induced by halothane, isoflurane, and thiamylal

Drugs and doses (mg/kg)		Duration of LORR (s)		
		Halothane	Isoflurane	Thiamylal
GABA uptake inhibitor				
Saline		93.5 ± 7.4 (11)	31.0 ± 0.8 (11)	1328.6 ± 316.3 (8)
Nipecotic acid	200	87.5 ± 7.8 (8)	28.3 ± 3.2 (8)	1637.5 ± 478.8 (8)
	400	94.5 ± 6.2 (8)	33.5 ± 1.5 (8)	1325.0 ± 261.3 (8)
NO-711	1	108.0 ± 21.2 (10)	38.4 ± 9.0 (10)	2067.1 ± 467.7 (9)
	2	108.5 ± 10.1 (10)	46.9 ± 7.0 (10)	3543.9 ± 738.9* (10)
Guvacine	10	96.9 ± 11.5 (10)	35.5 ± 4.1 (10)	1270.9 ± 215,1 (9)
	20	118.8 ± 22.6 (10)	39.2 ± 3.9 (10)	1501.5 ± 283.3 (10)
SKF89976A	1	104.1 ± 14.4 (10)	52.8 ± 12.8 (10)	1580.0 ± 426.4 (9)
	2	135.0 ± 29.3 (10)	41.4 ± 5.3 (10)	3884.0 ± 935.8* (10)
GABA mimetic				
Saline		93.3 ± 6.6 (10)	48.2 ± 8.6 (10)	908.5 ± 200.4 (11)
Muscimol	0.5	324.6 ± 49.8 (10)	235.6 ± 51.4 (10)	6109.3 ± 1292.1**
	1	859.5 ± 137.5** (10)	648.1 ± 205.3** (8)	10000.0 ± 0.0** (9)
Saline		87.6 ± 6.4 (11)	48.2 ± 8.6 (10)	608.0 ± 117.2 (10)
Baclofen	10	209.3 ± 27.2 (11)	129.5 ± 17.2 (8)	5730.0 ± 648.6** (10)
	20	485.1 ± 107.4** (10)	524.6 ± 211.7** (10)	8238.0 ± 700.9** (10)
Saline		89.4 ± 6.7 (11)	48.2 ± 8.6 (10)	646.6 ± 111.1 (11)
Diazepam	1	210.4 ± 48.1* (10)	63.3 ± 10.1 (9)	3272.7 ± 621.2** (11)
	2	208.6 ± 19.1* (10)	92.5 ± 13.1* (8)	4446.0 ± 423.7** (10)
GABA antagonist				
Saline		90.5 ± 11.2 (11)	39.7 ± 5.3 (9)	908.5 ± 200.4 (11)
Picrotoxin	1	97.8 ± 17.6 (10)	31.2 ± 5.6 (10)	780.8 ± 185.3 (8)
	2	85.5 ± 9.7 (10)	33.0 ± 1.5 (8)	403.6 ± 48.4 (8)
Saline		88.8 ± 9.8 (11)	39.7 ± 5.3 (9)	908.5 ± 200.4 (11)
Bicuculline	1	73.9 ± 7.8 (11)	30.4 ± 2.8 (10)	962.6 ± 232.4 (9)
	2	86.3 ± 10.3 (13)	30.8 ± 5.5 (8)	851.7 ± 177.3 (9)
	4	83.0 ± 10.3 (13)	30.5 ± 1.3 (8)	681.7 ± 121.2 (9)

Values are means ± S.E.M. Numbers in parentheses represent the number of animals.
* $P < .05$ vs. saline control.
** $P < .005$ vs. saline control.

thiamylal and 2 mg/kg for isoflurane). However, $GABA_A$ receptor antagonists, picrotoxin and bicuculline, at the doses tested had no effect on the duration of LORR induced by these anesthetics, except that picrotoxin (2 mg/kg) shortened the thiamylal-induced sleeping time, but this was not significantly different from controls.

4. Discussion

Numerous attempts have been made to elucidate the involvement of the GABAergic system in the mechanisms of anesthesia. In vitro studies have clearly demonstrated that various types of anesthetic and hypnotic agents including volatile anesthetics, barbiturates, and benzodiazepines potentiate GABAergic synaptic transmission at least by activation/potentiation of $GABA_A$ receptor (see reviews Franks and Lieb, 1998; Little, 1996; Tanelian et al., 1993). Li and Pearce (2000) showed that halothane slows the dissociation of GABA from $GABA_A$ receptor, resulting in the prolongation of the inhibitory postsynaptic current (IPSC) decay at central synapses, which was suggested to be a major factor in the production of anesthesia. However, the precise mechanisms of the pre- and postsynaptic actions of anesthetic agents on the GABAergic system underlying anesthesia were not fully clarified, and there is little evidence from in vivo investigations on the interaction of anesthetics with the GABAergic system. To address this issue, the present study examined the relationship between changes in GABAergic activities by pharmacological manipulation in the central nervous system and anesthetic effect of volatile and intravenous anesthetics in an in vivo behavioral model in mice.

Consistent with previous studies demonstrating the effects of barbiturates and benzodiazepines on anesthesia in vivo (Chambers et al., 1978; Chweh et al., 1987; Geller et al., 1989; Moody and Skolnick, 1988; Sivam et al., 1982), the present study demonstrated that the $GABA_A$ receptor agonists, muscimol, as well as the benzodiazepine receptor agonist, diazepam, shortened the onset of LORR and prolonged the duration of LORR induced by volatile and intravenous anesthetics, while $GABA_A$ receptor antagonists reversed the anesthetics action in some cases. These results suggested that GABA receptor agonists and antagonists

conversely modified anesthetics-induced LORR. Furthermore, GABA uptake inhibitors permeable to the BBB, but not those less permeable to the BBB, accelerated the onset of LORR induced by anesthetics. In addition, the selective $GABA_B$ receptor agonist, baclofen (Bowery, 1993), shortened the onset of and prolonged the duration of LORR induced by all anesthetics examined in the present study. $GABA_B$ receptor can be distinguished pharmacologically from $GABA_A$ receptor (Bowery, 1993) and a recent molecular biological approach successfully identified $GABA_B$ receptor cDNA, which is a member of gene family of G protein-coupled receptor with the 7 transmembrane structure (Marshall et al., 1999). Activation of $GABA_B$ receptor is known to cause the inhibition of Ca^{2+} channels and the activation of K^+ channels, resulting in presynaptic inhibition (Bowery, 1993). Hirota and Roth (1997) demonstrated that sevoflurane at clinical concentrations activates both $GABA_A$- and $GABA_B$-mediated inhibitions in area CA1 of the rat hippocampus. Taken together, it is suggested that anesthesia induced by volatile and intravenous anesthetics might be correlated with the modification of pre- and/or postsynaptic GABAergic activities.

However, the current results do not provide any clear distinction between pre- and postsynaptic events necessary for anesthesia induced by volatile and intravenous anesthetics. The effects of GABA uptake inhibitors on halothane-, isoflurane-, and thiamylal-induced LORR suggest, at least in part, the importance of presynaptic GABAergic activity in the anesthetic state induced by these volatile and intravenous anesthetic agents. If the effect on the GABA uptake system is involved in the presynaptic modulation of GABAergic activities produced by anesthetics, it should be requisite to provide evidence that they do affect the GABA uptake. However, the effects of general anesthetics on the GABA uptake process are not thoroughly understood. A previous study reported no effect of thiopental, methohexital, ketamine, halothane, or urethane on the uptake of GABA into rat brain slices (Minchin, 1981), while another reported a concentration-dependent inhibition of GABA uptake into rat striatal synaptosomes by propofol, etomidate, thiopental, and ketamine, but not by halothane or enflurane (Mantz et al., 1995). We have strictly examined the effects of the volatile anesthetics, halothane and isoflurane, and the intravenous anesthetic, thiamylal, on GABA uptake in COS cells transfected with mouse GABA transporter (mGAT-1) cDNA and in rat brain synaptosomes, and found that both halothane and isoflurane, but not thiamylal, at clinically relevant concentrations inhibited GABA uptake in both preparations (Sugimura et al., 2001). Provided that this presynaptic mechanism is involved in the production of anesthesia, further manipulation of the same pathways would not yield an appreciable affect. Such an idea appears to be in favor of accounting for the present observation that the pretreatment with GABA uptake inhibitors shortened the onset of LORR, but failed to affect the duration of LORR induced by halothane or isoflurane, while they shortened the onset of and prolonged the duration of LORR induced by thiamylal. Although the studies examining the effects of GABAergic agents on anesthesia induced by volatile and intravenous anesthetics in an in vivo behavioral model in mice used here do have clear limitations in interpretation, taken together with our previous observation of the effects of anesthetics on GABA uptake in in vitro systems (Sugimura et al., 2001), it is suggested that possible sites in the presynaptic GABA uptake system in addition to postsynaptic GABA receptors might be involved in the anesthetic action.

The noncompetitive antagonist, picrotoxin, prolonged the onset of LORR induced by the volatile anesthetics, halothane and isoflurane, and the intravenous anesthetic, thiamylal, whereas the competitive antagonist, bicuculline, only prolonged the onset of LORR induced by halothane. The latter finding may, at least in part, reflect the different mechanisms underlying anesthesia induced by volatile and intravenous anesthetics. Hirota et al. (1998) reported that the action of volatile and intravenous anesthetics on evoked synaptic responses are different, which may result from different effects on GABAergic mechanisms that modulate synaptic transmission, as suggested by the different sensitivities to bicuculline. However, bicuculline slowed the onset of LORR induced by halothane but not by isoflurane. The present results were inconsistent with such an interpretation. It is not known at present why the different antagonists have markedly different effects. Further studies are needed to clarify the effects of bicuculline and picrotoxin on anesthesia induced by volatile and intravenous anesthetics.

In conclusion, the present study examined the in vivo effects of GABA receptor modulators and GABA uptake inhibitors on volatile and intravenous anesthetic-induced anesthesia in mice as a behavioral model. Although the present study has clear limitations in interpretation, it is suggested that anesthesia induced by these anesthetics might be correlated with the modification of the pre- and/or postsynaptic GABAergic activities.

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