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Original Article

Lidocaine may contribute to general anesthesia by different mechanisms in mammalian central nervous system

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Abstract: Background: Systemic administration of local anesthetics can produce systemic actions, but the details of these actions and mechanisms are still unclear. We evaluated the systemic actions of three local anesthetics and their underlying mechanisms in this study. Methods: Lidocaine, bupivacaine and ropivacaine were injected into C57BL/6 mice through tail vein, alone or together with hyperpolarization-activated cyclic nucleotide-gated (HCN) channels blocker ZD 7288 or N-methyl-D-aspartic acid (NMDA) receptor blocker MK-801, and hypnotic actions of these local anesthetics, were evaluated by the length of loss of righting reflex (LORR). Results: All of these three local anesthetics directly produced hypnotic actions with half effective dose (ED_{50}) of 19.0±2.4, 4.3±0.2 and 4.2±0.4 mg/kg. The effects of lidocaine on LORR were enhanced by ZD 7288, ED_{50} from 19.0±2.4 mg/kg to 14.1±1.0 mg/kg (P<0.01) or to 13.1±0.8 mg/kg (P<0.01) by MK-801, respectively. The effects of ropivacaine on LORR were weakened by ZD 7288 or MK-801, with ED_{50} from 4.2±0.4 mg/kg to 6.9±0.3 mg/kg (P<0.01) or to 6.7±0.2 mg/kg (P<0.01). ZD 7288 could weaken the effects of bupivacaine on LORR with ED_{50} from 4.3±0.2 mg/kg to 5.6±0.4 mg/kg (P<0.01), while MK-801 had no influence on it with ED_{50} from 4.3±0.2 mg/kg to 4.5±0.5 mg/kg (P>0.05). Conclusion: HCN channels and NMDA receptor may play an important role in the systemic actions of local anesthetics. The mechanisms of effects of lidocaine on the mammalian central nervous system may be different from those of bupivacaine and ropivacaine.

Keywords: General anesthesia, lidocaine, bupivacaine, ropivacaine, ZD 7288, MK-801

Introduction

Since cocaine was applied firstly in ophthalmic surgery by Koller in 1884 [1], local anesthetics, such as lidocaine and bupivacaine, have been widely used in clinic. Systemic administrations of local anesthetics combined with general anesthetics by intravenous application have been exploited clinically for a long time [2]. It has been demonstrated that systemic administration of low-dose lidocaine by intravenous injection can enhance the effects of general anesthesia, such as antiarrhythmia [3], analgesic [4, 5], anti-hyperalgesic [5, 6], suppressing of postoperative pain [5, 7], decreasing of opiate anesthetics requirements, decreasing of minimal alveolar concentration (MAC) of inhalational anesthetics during general anesthesia [8, 9] and so on. However, the underlying mechanism of synergistic interactions between local anesthetics and general anesthetics are still unclear.

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels and N-methyl-D-aspartic acid (NMDA) receptor were indicated as the molecular targets of general anesthetics [10]. Previous studies indicated that regional anesthetic effects of lidocaine and ropivacaine were contributed by HCN channel [11-14]. In addition, forebrain HCN1 channel contributed to hypnotic actions of ketamine, an NMDA recep-

tors inhibitor [10]. In this study, we explored the hypnotic actions and possible mechanisms of systemic actions of lidocaine, bupivacaine and ropivacaine. The results will help to design novel general anesthetic drugs.

Materials and methods

Animals

In this study, 2-4 month old male adult wild-type C57BL/6 mice, weighted 18.5-31.5 g, were used. The study was approved by the institutional Animal Experimentation Ethics Committee of Sichuan University (Chengdu, Sichuan, China). These mice were housed for four to six per cage and fed in controlled condition (temperature 19.5-24.5°C and relative humidity of 45 to 65%) on a standard 12 h light/dark cycle (lights on, 6:00 AM; lights off, 6:00 PM). Free access to food and water was maintained throughout the study period. All the mice were acclimated in cages at least 5 days before experiments.

Experimental protocol

The study complied with random group design. All of the animals were assigned randomly to treatment groups. The sample size of this study is 8 mice per dose group. Behavioral experiments were performed from 8:00 AM to 2:00 PM. To obtain dose-response curves of hypnosis, lidocaine (9-25 mg/kg), bupivacaine (1.5-6.5 mg/kg), or ropivacaine (1-7 mg/kg) were injected via the tail vein of the mice. For underlying mechanisms exploration and obtaining of dose-response curves, different doses of lidocaine, bupivacaine and ropivacaine were injected combined with ZD 7288 (HCN channels blocker, 25 mg/kg) or MK-801 (NMDA receptor blocker, 25 mg/kg). In our pre-test, systemic administration of ZD 7288 (25 mg/kg) or MK-801 (25 mg/kg) were all failed to induce reversible loss of righting reflex (LORR) in mice. So here, MK-801 (25 mg/kg) or ZD 7288 (25 mg/kg) was administered 10 min before the injection of each local anesthetics. A flashback of blood appearing during aspiration indicates a successful injection. Each animal was used only once for the intravenous injection of a single drug or a combination of two different drugs (combination of local anesthetics with ZD 7288 or MK-801). All drugs were administered under 0.05 ml at suitable concentration. All drugs were purchased from Sigma-Aldrich Co. Ltd. (Shanghai, China).

Analysis of hypnotic action in mice

The length of LORR was utilized to represent hypnosis, and an effective LORR should occur within 10 s after injection and last for at least 10 s [15]. To be succinct, the sedative level was recorded according to the following scale: 0 = a normal righting reflex; +1 = the righting time was within 2 s (slightly impaired righting reflex); +2 = the righting time was >2 s but <10 s (moderately or severely impaired righting reflex; +3 = no righting within 10 s (losing righting reflex). The sedative scores were expressed as mean (95% confidence interval) and used to evaluate the hypnotic actions of each local anesthetic in mice, with or without ZD 7288 or MK-801. The time between LORR (shown as a score of +3) and the state mice regained the ability to right themselves (shown as a score of +2) were recorded as the LORR duration (seconds).

Data acquisition and analysis

Results were presented as mean ± SEM. SPSS 16.0 (SPSS Inc., Chicago, IL) was used. For LORR duration data, we used one-way analysis of variance (ANOVA) followed by post hoc of Turkey to analyze the significance of the difference among groups. We used Prism 6.0 (GraphPad, San Diego, CA) as Karber method to obtain ED_{50} for LORR and compared with one-way analysis of variance (ANOVA) with post hoc of Turkey. The data of sedative score were calculated as the mean with 95% confidence interval, and compared with non-parametric test. Significance was considered statistically as $P<0.05$ and statistically highly significant as $P<0.01$.

Results

Intravenous injection of local anesthetics produced LORR

Systemic administration of lidocaine (9-25 mg/kg) induced LORR in a dose dependent fashion with an ED_{50} of 19.0±2.4 mg/kg (n=8) (**Figure 1A**), corresponding to the sedative score (from 0.63 to 3 at dose of 15, 18.5, 22, 25 mg/kg, **Table 1**) and LORR duration (from 0 to 132.5±35.5 s, mean ± SEM, **Figure 1B**). Ropivacaine and bupivacaine produced LORR

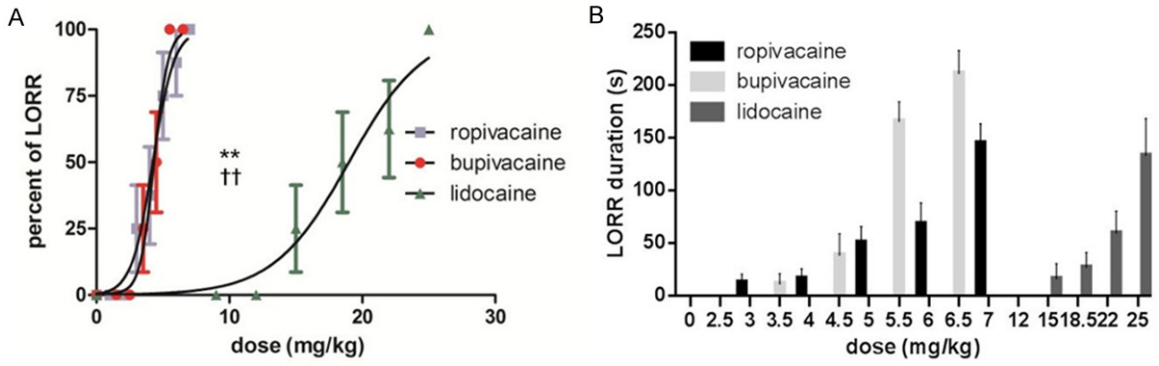

Figure 1. LORR induced by local anesthetics in mice. Different concentrations of lidocaine (12-25 mg/kg), bupivacaine (1.5-6.5 mg/kg) and ropivacaine (1-7 mg/kg) were injected into mice intravenously. A. Percent of LORR (ED_{50}). B. LORR durations. (**P<0.01 ropivacaine vs. lidocaine; ††P<0.01 bupivacaine vs. lidocaine) (n=8).

Table 1. Hypnotic actions of lidocaine, bupivacaine and ropivacaine

Agents	Dose (mg/kg)	Sedative scores
Lidocaine	15	0.63 (-0.37-1.62)
Lidocaine	18.5	1.5 (0.16-2.84)
Lidocaine	22	1.88 (0.58-3.17)
Lidocaine	25	3
Ropivacaine	3	0.75 (-0.41-1.91)
Ropivacaine	4	1.13 (-0.17-2.42)
Ropivacaine	5	2.25 (1.09-3.41)
Ropivacaine	6	2.63 (1.74-3.51)
Ropivacaine	7	3
Bupivacaine	3.5	0.63 (-0.37-1.62)
Bupivacaine	4.5	1.5 (0.16-2.84)
Bupivacaine	5.5	3
Bupivacaine	6.5	3

Sedative scores were determined by LORR and expressed as mean (95% confidence interval). The third column shows the sedative scores of lidocaine, bupivacaine or ropivacaine for each dose (n=8).

in a significantly lower dose with an ED_{50} of 4.3±0.2 mg/kg and 4.2±0.4 mg/kg, respectively (**Figure 1A**), corresponding to the sedative score (from 0.75 to 3 at dose of 3, 4, 5, 6, 7 mg/kg for ropivacaine, from 0.63 to 3 at dose of 3.5, 4.5, 5.5, 6.5 mg/kg for bupivacaine, **Table 1**) and LORR duration (from 0 to 144.5±18.5 s for ropivacaine at dose from 1 to 7 mg/kg, from 0 to 210.3±22.4 s for bupivacaine at dose from 1.5 to 6.5 mg/kg, **Figure 1B**). No significant difference in ED_{50} of LORR was found between bupivacaine and ropivacaine (P>0.05), but lidocaine and bupivacaine (P<0.01), lidocaine and ropivacaine (P<0.01).

MK-801 or ZD 7288 extended the LORR duration of lidocaine

In our pre-test, MK-801 was unable to induce LORR alone at the dose of 25 mg/kg. The ED_{50} of lidocaine LORR decreased from 19.0±2.4 mg/kg to 13.1±0.8 mg/kg (P<0.01) (**Figure 2A**) when lidocaine was administrated together with MK-801 (25 mg/kg). MK-801 also changed the sedative score of lidocaine from 0 to 1.13 (12 mg/kg), 0.63 to 2.25 (15 mg/kg) and 1.5 to 3 (18.5 mg/kg) (**Table 2**). MK-801 extended the LORR duration of lidocaine (18.5 mg/kg) from 26.3±14.7 s to 65.0±6.8 s (P<0.01) (**Figure 2B**).

In our pre-test, ZD 7288 (25 mg/kg) failed to induce LORR alone. The ED_{50} of lidocaine LORR decreased from 19.0±2.4 mg/kg to 14.1±1.0 mg/kg (P<0.01) when administrated together with ZD 7288 (**Figure 2A**). ZD 7288 also changed the sedative score of lidocaine, from 0 to 1.13 (12 mg/kg), 0.63 to 1.5 (15 mg/kg) and 1.5 to 3 (18.5 mg/kg) (**Table 2**), and extended the LORR duration of lidocaine (22 mg/kg) from 58.75±21.3 s to 116.6±19.7 s (P<0.01) (**Figure 2B**).

MK-801 or ZD 7288 decreased the LORR duration of ropivacaine

When ropivacaine was injected into the mice after the injection of ZD 7288 (25 mg/kg) or MK-801 (25 mg/kg), there was significant increase of ED_{50} of ropivacaine LORR from 4.2±0.4 mg/kg to 6.9±0.3 mg/kg (ZD 7288, P<0.01) and to 6.6±0.4 mg/kg (MK-801, P<0.01) (**Figure 3A**). ZD 7288 decreased the

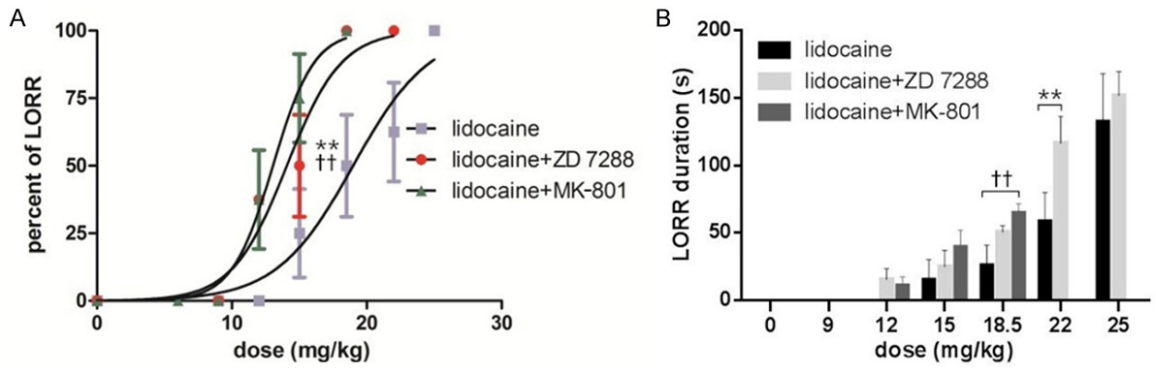

Figure 2. LORR induced by lidocaine with or without ZD 7288 or MK-801 in mice. Different concentrations of lidocaine (12-25 μg/kg), with or without ZD 7288 (25 mg/kg) or MK-801 (25 μg/kg), were injected into mice intravenously. A. Percent of LORR (ED_{50}). B. LORR durations. (***P*<0.01 lidocaine vs. lidocaine+ZD 7288; ††*P*<0.01 lidocaine vs. lidocaine+MK-801) (n=8).

Table 2. Hypnotic actions of lidocaine, lidocaine+ZD 7288 and lidocaine+MK-801

Agents	Sedative scores			
	12 (mg/kg)	15 (mg/kg)	18.5 (mg/kg)	22 (mg/kg)
Lidocaine	0	0.63 (-0.37-1.62)	1.5 (0.16-2.84)	1.88 (0.58-3.17)
Lidocaine+ZD 7288	1.13 (-0.17-2.42)	1.5 (0.16-2.84)	3*	3
Lidocaine+MK-801	1.13 (-0.17-2.42)	2.25 (1.09-3.41)*	3*	

Sedative scores of lidocaine were determined by LORR and expressed as mean (95% confidence interval). The second column "sedative scores" shows the results of each exact dose of lidocaine with or without ZD 7288 (25 μg/kg) or MK-801 (25 μg/kg). **P*<0.05 vs. lidocaine group; ***P*<0.01 vs. lidocainegroup (n=8).

sedative score of ropivacaine, from 2.25 to 0 (5 mg/kg), 2.63 to 0.63 (6 mg/kg) and 3 to 1.5 (7 mg/kg) (**Table 3**), and also the LORR duration of ropivacaine (7.0 mg/kg) from 144.5±18.5 s to 22.0±9.4 s (*P*<0.01) (**Figure 3B**). MK-801 decreased the sedative score of ropivacaine, from 2.25 to 0 (5 mg/kg), 2.63 to 0.63 (6 mg/kg), 3 to 1.88 (7 mg/kg) (**Table 3**), and the LORR duration of ropivacaine (7.0 mg/kg) from 144.5±18.5 s to 43.9±15.8 s (*P*<0.01) (**Figure 3B**).

ZD 7288 decreased the LORR duration of bupivacaine

When bupivacaine was administrated after the injection of ZD 7288 (25 mg/kg), there was a significant increase of ED_{50} of bupivacaine from 4.3±0.2 mg/kg to 5.6±0.4 mg/kg (*P*<0.01) (**Figure 4A**). ZD 7288 decreased the sedative score of bupivacaine, from 0.63 to 0 (3.5 mg/kg), 1.5 to 0.75 (4.5 mg/kg), 3 to 1.13 (5.5 mg/kg) (**Table 4**), and the LORR duration of bupivacaine (6.5 mg/kg) from 210.3±22.4 s to 140.0±21.5 s (*P*<0.05) (**Figure 4B**).

When a series of doses of bupivacaine were injected together with MK-801, there was no significant change of ED_{50} (**Figure 4A**), from 4.3±0.2 mg/kg to 4.5±0.5 mg/kg (*P*>0.05). Furthermore, MK-801 did not change either the sedative score (**Table 4**), or the LORR duration of bupivacaine (6.5 mg/kg), from 210.3±22.4 s to 167.3±19.6 s (*P*>0.05) (**Figure 4B**).

Discussion

Local anesthetics are well believed to target sodium channels [16]. Previous studies have demonstrated that lidocaine targeted the HCN channels [12] and NMDA receptors [17]. Local anesthetics have also been applied in general anesthesia. In this study, we found that three local anesthetics, lidocaine, bupivacaine and ropivacaine, can produce reversible LORR with different ED_{50}. MK-801 (NMDA receptor inhibitor) and ZD 7288 (HCN channels blocker) can enhance the effects of lidocaine on reversible LORR, but not bupivacaine and ropivacaine, in mice. Lidocaine alone was reported to be capa-

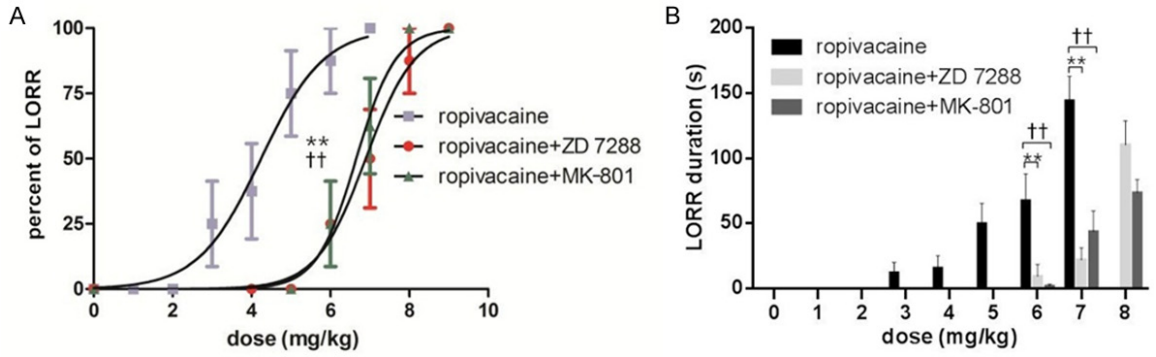

Figure 3. LORR induced by ropivacaine with or without ZD 7288 or MK-801 in mice. Different concentrations of ropivacaine (1-7 mg/kg), with or without ZD 7288 (25 μg/kg) or MK-801 (25 μg/kg), were injected into mice intravenously. A. Percent of LORR (ED_{50}). B. LORR durations. (**P<0.01 ropivacaine vs. ropivacaine+ZD 7288; ††P<0.01 ropivacaine vs. ropivacaine+MK-801) (n=8).

Table 3. Hypnotic actions of ropivacaine, ropivacaine+ZD 7288 and ropivacaine+MK-801

Agents	Sedative scores			
	4 (mg/kg)	5 (mg/kg)	6 (mg/kg)	7 (mg/kg)
Ropivacaine	1.13 (-0.17~2.42)	2.25 (1.09~3.41)	2.63 (1.74~3.51)	3
Ropivacaine+ZD 7288	0	0**	0.63 (-0.37~1.62)**	1.50 (0.61~2.84)*
Ropivacaine+MK-801	0	0**	0.63 (-0.37~1.62)**	1.88 (0.58~3.17)

Sedative scores of ropivacaine were determined by LORR and expressed as mean (95% confidence interval). The second column "sedative scores" shows the results of each exact dose of ropivacaine with or without ZD 7288 (25 μg/kg) or MK-801 (25 μg/kg). *P<0.05 vs. ropivacaine group; **P<0.01 vs. ropivacaine group (n=8).

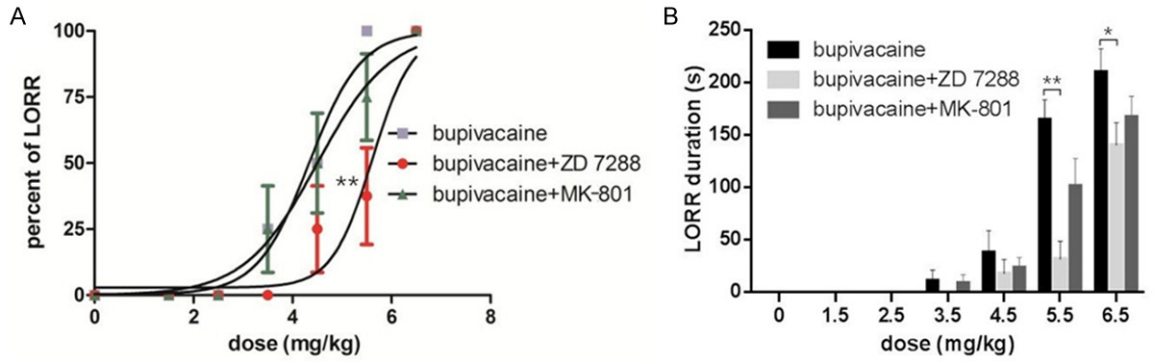

Figure 4. LORR induced by bupivacaine with or without ZD 7288 or MK-801 in mice. Different concentrations of bupivacaine (1.5-6.5 mg/kg), with or without ZD 7288 (25 μg/kg) or MK-801 (25 μg/kg), were injected into mice intravenously. A. Percent of LORR (ED_{50}). B. LORR durations. (*P<0.05 bupivacaine vs. bupivacaine+ZD 7288; **P<0.01 bupivacaine vs. bupivacaine+ZD 7288) (n=8).

ble of decreasing bispectral index (BIS) to 0 in human [18]. Also, the ED_{50} of bupivacaine and ropivacaine were lower than the dose of cardiovascular system toxicity [19]. These results indicated lidocaine might contribute to general anesthesia by different mechanisms in mammalian central nervous, compared with bupivacaine and ropivacaine.

Although local anesthetics have been applied in clinic with general anesthetics for a long time, its mechanism remains unclear. It was generally believed that the related actions induced by traditional local anesthetics were related to the blockage of nervous impulse by blocking sodium channels [16]. The ED_{50} of tetrodotoxin (TTX) for blocking the Nav1.2 is

Table 4. Hypnotic Actions of Bupivacaine, Bupivacaine+ZD 7288 and Bupivacaine+MK-801

Agents	Sedative scores			
	3.5 (mg/kg)	4.5 (mg/kg)	5.5 (mg/kg)	6.5 (mg/kg)
Bupivacaine	0.63 (-0.37~1.62)	1.5 (0.16~2.84)	3	3
Bupivacaine+ZD 7288	0	0.75 (-0.41~1.91)	1.13 (-0.17~2.42)**	3
Bupivacaine+MK-801	0.63 (-0.37~1.62)	1.5 (0.16~2.84)	2.25 (1.09~3.41)	3

Sedative scores of bupivacaine were determined by LORR and expressed as mean (95% confidence interval). The second column "sedative scores" shows the results of each exact dose of bupivacaine with or without ZD 7288 (25 μg/kg) or MK-801 (25 μg/kg). *P<0.05 vs. bupivacaine group; **P<0.01 vs. bupivacaine group (n=8).

about 10 nM [20], and Nav1.2 is widely expressed in central nervous system (CNS). ED_{50} of lidocaine on sodium channels is about 100 μM [21]. This comparison indicates that potency of TTX is at least 10,000-fold higher than that of lidocaine on sodium channels. However, TTX did not produce LORR or show any sign of sedation at the dosage of 6 mg/kg [22], while the ED_{50} of lidocaine in the present study is 19.0 mg/kg, about 3,000-fold higher than TTX. Thus, blockage of sodium channels could not completely explain the sedative effect of lidocaine, and there might be different mechanisms of sedative effect of local anesthetics. To better understand such mechanisms, we investigated the roles of HCN channels and NMDA receptors in hypnotic actions of local anesthetics by using ZD 7288 and MK-801 as valuable tools.

HCN channels have been discovered over the past decades [23, 24]. In mammals, the HCN channel family comprises four members (HCN1-HCN4) [23], and they are widely expressed in various tissues. Our study confirmed that ZD 7288, as a HCN channel blocker, could enhance the reversible LORR effect of lidocaine in mice. Although previous studies indicated that ZD 7288 could enhance the local anesthetic effects of lidocaine and ropivacaine [14, 25], however, we found in this study that ZD 7288 could only enhance the hypnotic actions of lidocaine but not bupivacaine or ropivacaine.

NMDA receptors are expressed in the brain and play an important role in excitatory synaptic transmission and excitotoxicity in the CNS [26]. Previous studies indicated that local anaesthetics could inhibit the activation of human NMDA receptors [27]. In this study, we observed the enhanced effects of lidocaine on LORR by NMDA receptor blocker MK-801, which indicated that NMDA receptors might contribute to the hypnotic action of lidocaine.

To our surprise, instead of enhancing the ropivacaine effects on LORR, systemic application of NMDA blocker MK-801 and HCN channel blocker ZD 7288 weakened such effects. Meanwhile, ZD 7288 was also observed to weaken the hypnotic action of bupivacaine. However, MK-801, the specific blocker of NMDA receptor, failed to alter the LORR induced by bupivacaine. We speculated that ZD 7288 and MK-801 might aim at some targets the same as ropivacaine, consequently competitively bind to these targets to weaken the effects of ropivacaine on LORR. Thus, the mechanisms of systemic actions of lidocaine, ropivacaine and bupivacaine might be different in the mammalian central nervous system.

HCN channels and NMDA receptor were demonstrated to contribute to systemic actions of local anesthetics. These results provide better understand of the systemic action of local anesthetics and their underlying mechanisms. This study also indicated targets for developing new anesthesia adjuvant drugs, and lidocaine might be the lead compound.

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Disclosure of conflict of interest

None.

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