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Midazolam in rabbits terminates dysrhythmias caused by intracerebroventricular ropivacaine*

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Abstract: The current study was designed to investigate the mechanisms by which ropivacaine may act within the central nervous system (CNS) to produce cardiotoxicity. Eighty New Zealand rabbits were divided into four groups randomly. In Group 1, 20 rabbits received intracerebroventricular (icv) saline, and then received icv ropivacaine 30 min later. In Group 2, 20 rabbits received icv ropivacaine. Whenever dysrhythmias continued for more than 5 min, 0.1 ml saline was administered into the left cerebral ventricle. Ten minutes later, 0.1 ml midazolam was given into the left lateral ventricle. In Group 3, 20 rabbits received icv ropivacaine, and once the dysrhythmias developed, the inspired isoflurane concentration was increased from 0.75% to 1.50%. In Group 4, 20 animals received an intravenous (iv) phenylephrine infusion until dysrhythmias occurred. In Group 1, the rabbits did not develop dysrhythmias in response to icv saline, whereas dysrhythmias did develop in these animals after icv ropivacaine. In Group 2, icv saline had no effect on the dysrhythmias; however, icv midazolam terminated cardiac dysrhythmias. In Group 3, an increase in the concentration of the inspired isoflurane had no effect on dysrhythmias. In Group 4, icv midazolam had no effect on dysrhythmias in response to iv phenylephrine. Ropivacaine administered directly into the CNS is capable of producing cardiac dysrhythmias; midazolam terminated dysrhythmias presumably by potentiation of γ-aminobutyric acid (GABA) receptor activity. Our results suggest that ropivacaine produces some of its cardiotoxicity not only by the direct cardiotoxicity of the drug, but also by the CNS effects of ropivacaine.

Key words: Ropivacaine, Cardiotoxicity, Ventricular cerebrospinal fluid, Central nervous system (CNS), Dysrhythmias, Midazolam

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1 Introduction

Most studies (Mather, 2010; Kuthiala and Chaudhary, 2011) of ropivacaine cardiotoxicity have focused on the direct myocardial effect of ropivacaine as the mechanism producing cardiotoxicity. However, there is no evidence that ropivacaine may produce cardiotoxicity in part by drug actions within central nervous system (CNS). We developed a rabbit model, in which cardiac dysrhythmias resulted from intracerebroventricular (icv) ropivacaine administration. Based on our results and a review of the relevant literature (Bilir *et al.*, 2006; Shen *et al.*, 2010), we propose a model to explain the mechanism by which icv ropivacaine acts within the CNS to produce cardiotoxicity.

2 Animals and methods

2.1 Animals

Eighty New Zealand male rabbits were purchased from the Baishiyi Experimental Rabbit Farm, Chongqing, China. The experimental protocol was

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approved by the Animal Subjects Review Committee of the Medical School of Xi'an Jiaotong University.

2.2 Methods and procedures

All animals (weight (3.0±2.12) kg, age (8±0.5) months) were anesthetized with isoflurane in O_2. After tracheal intubation, the lungs were ventilated with a Harvard pump. Expired CO_2 was continuously monitored (Beckman LB-2 medical gas analyzer). Ventilation was adjusted to maintain normocapnia. The right femoral artery was cannulated for blood pressure monitoring and blood sampling. The right femoral vein or the ear vein was cannulated for venous access.

The head was secured with blunt prongs in a stereotactic frame and the skull was exposed through a longitudinal scalp incision. A 2-mm burr hole was drilled at a point 5 cm left of the sagittal suture and 5 cm caudad to the coronal suture. A 20-G needle was inserted through the hole into the left lateral ventricle. A T-piece connected to this needle allowed simultaneous perfusion of the ventricle and measurement of ventricular cerebrospinal fluid (CSF) pressure. Continuous measurement of ventricular CSF pressure allowed selection of a rate of icv drug administration that did not increase CSF pressure.

The icv perfusion was established by perfusing mock CSF (pH 7.33; 300 mOsmol/kg) through the ventricle and was considered successful if CSF pressure did not increase above pre-perfusion values and mock CSF flowed from the cisternal cannula. The icv perfusion was controlled by a syringe pump at a rate of 0.01 ml/min.

Needle electrodes were inserted at both shouders and both thighs to monitor the electrocardiogram (ECG). Mean artery pressure (MAP), heart rate (HR), CSF pressure, and end-tidal CO_2 were continuously recorded. After completion of surgical preparation, isoflurane was decreased to 0.75% inspired concentration (measured by Beckman LB-2, Drager Co., Ltd., America) and paralysis was initiated by continuous infusion of pancuronium bromide (40 μg/h) through the venous cannula. After the change in anesthetic, we allowed at least 30 min to elapse before beginning any experiments. A simplified outline of the experimental groups and treatments is presented in Table 1. The animals were randomly divided into four groups.

Table 1 Experimental groups and treatments

Group	Sequential treatment		
	1st	2nd	3rd
1	icv saline	icv ropivacaine	
2	icv ropivacaine	icv saline	icv midazolam
3	icv ropivacaine	Increased inspired isoflurane	
4	iv phenylephrine	icv midazolam	

1. Group 1. In 20 of the 80 rabbits, studies were performed to determine whether the cardiac dysrhythmias that result from icv ropivacaine are the result of ropivacaine or the result of saline, with which ropivacaine was diluted from 1.0% to 0.5%. In addition, we sought to determine the duration of dysrhythmias induced by icv ropivacaine. All 20 rabbits received 0.1 ml boluses of saline (300 mOsmol/kg solvent; pH 7.0) through the ventricular cannula at 5 min intervals to a total dose of 0.5 ml. MAP, HR, CSF pressure, ECG, and bispectral index (BIS) were monitored continuously. Thirty minutes later, these 20 rabbits received 0.5% ropivacaine at a rate of 0.01 ml/min (50 μg/min) (300 mOsmol/kg; pH adjusted to 7.0 by addition of 0.1 mol/L NaOH) until cardiac dysrhythmias developed. At the onset of cardiac dysrhythmias, 3 ml arterial blood was withdrawn for later ropivacaine analysis. Dysrhythmias were defined as any abnormal ventricular rhythm (e.g., ectopic beats occurring more often than five times per minute, bigeminy, trigeminy, or ventricular tachycardia).

2. Group 2. Twenty rabbits were surgically prepared as described above, and continuous icv perfusion was administered with 0.5% ropivacaine at a rate of 0.01 ml/min (50 μg/min) (300 mOsmol/kg; pH adjusted to 7.0 by addition of 0.1 mol/L NaOH). Perfusion was continued until cardiac dysrhythmias developed. The dose of ropivacaine that produced dysrhythmias was calculated as the product of the icv infusion rate and the duration of infusion. At the onset of cardiac dysrhythmias, 3 ml arterial blood was withdrawn for later ropivacaine analysis. Whenever dysrhythmias continued for more than 5 min, 0.1 ml saline (300 mOsmol/kg solvent; pH=7.0) was administered as a slow bolus (over 60 s) into the left cerebral ventricle. Midazolam was diluted by saline from 5000 to 10 μg/ml. Saline was administered as a pH, osmolality, and volume control for the

effects of icv midazolam (300 mOsmol/kg; pH adjusted to 7.0 by addition of 0.1 mol/L NaOH). This procedure was performed to determine whether the cardiac dysrhythmias that result from icv ropivacaine were terminated by midazolam or saline. Ten minutes after administration of saline, 0.1 ml midazolam (1 μg) was as a slow bolus (over 60 s) into the left lateral ventricle. Midazolam was administered to determine the effect of γ-aminobutyric acid (GABA) potentiation within the CNS on dysrhythmias produced by icv ropivacaine. MAP, HR, CSF pressure, and ECG were recorded continuously.

3. Group 3. Twenty rabbits were surgically prepared as described above and received icv 0.5% ropivacaine at 0.01 ml/min (50 μg/min) (300 mOsmol/kg; pH adjusted to 7.0 by addition of 0.1 mol/L NaOH) until dysrhythmias occurred. In animals in which dysrhythmias developed and continued for more than 5 min, the inspired isoflurane concentration was increased from 0.75% to 1.50%. This was performed in order to determine whether the effects of midazolam on dysrhythmias were specific to its ability to potentiate GABA within the CNS or whether these effects could be reproduced by any drug that produces nonspecific CNS depression. End-tidal isoflurane concentrations before and 3 min after the increase of the inspired isoflurane concentration were measured in these rabbits.

4. Group 4. Twenty animals were surgically prepared as described above and received an intravenous (iv) phenylephrine infusion (40 μg/ml) at a rate of 0.1 ml/min until dysrhythmias occurred. Five minutes after the dysrhythmias developed in the animals, 0.1 ml (1 μg) midazolam (300 mOsmol/kg; pH adjusted to 7.0 by addition of 0.1 mol/L NaOH) was injected into the lateral cerebral ventricle. This was done to determine whether CNS midazolam had the same effect on dysrhythmias produced by iv phenylephrine as it did on dysrhythmias produced by icv ropivacaine.

2.3 Ropivacaine analysis

Total ropivacaine in plasma samples was assayed by the method of high performance liquid chromatography (HPLC). The Agilent 1101 HPLC instrument was used with the column of Nova-Pak C_{18} (3.9 mm×150 mm). The mobile phase was composed of methyl alcohol-water at 2:1 (v/v). The flow rate was 1 ml/min. The fluorescence detective wave length was 230 nm.

2.4 Statistical analysis

Fisher's exact test was used to compare the effects of icv ropivacaine and saline on the incidence of dysrhythmias in rabbits receiving ropivacaine alone (Group 1). In rabbits receiving ropivacaine followed by midazolam, Fisher's exact test was used to compare the effects of icv saline and midazolam as treatments for dysrhythmias (Group 2). One-way analysis of variance (ANOVA) for repeated measures was used to compare MAP and HR after icv ropivacaine, after icv midazolam, and at the point that dysrhythmias recurred. A pooled *t*-test was used to compare differences in time from peak increase in MAP to onset of dysrhythmias between Groups 1, 2, 3, and 4. All results are reported as mean±standard deviation (SD). A *P* value of 0.05 was considered statistically significant.

3 Results

The general data and the baseline MAP, HR, and arterial blood gas were not significantly different among the four groups (Table 2).

Table 2 Comparison of the baseline conditions among the four groups before drug administration

Group	*n*	BW	MAP (mmHg)	HR (beat/min)	pH	$PaCO_2$ (mmHg)
1	20	3.0±2.10	86±10.9	257±23	7.41±0.02	29±3.4
2	20	3.1±1.96	88±11.1	265±19	7.40±0.01	30±3.5
3	20	3.0±1.98	91±9.6	255±33	7.42±0.01	31±3.0
4	20	3.0±2.02	87±9.5	260±26	7.39±0.03	29±2.9

Data are expressed as mean±SD. BW: body weight; MAP: mean artery pressure; HR: heart rate; $PaCO_2$: partial pressure of carbon dioxide in artery

3.1 Group 1

Group 1 did not develop cardiac dysrhythmias in response to icv saline, whereas cardiac dysrhythmias did develop in all 20 animals after icv ropivacaine (P=0.003). Dysrhythmias consisted of multifocal premature ventricular contractions, bigeminy, trigeminy, and brief runs of ventricular tachycardia. The average dose of ropivacaine that produced cardiac dysrhythmias was (833±159) μg ((0.167±0.032) ml), ropivacaine plasma concentrations averaged (0.23±0.09) μg/ml at the onset of dysrhythmias, and the time interval from icv ropivacaine to the onset of the first dysrhythmia was (16.9±3.2) min (Table 3). The duration of ropivacaine-induced dysrhythmias lasted (69±11) min.

Table 3 Comparison of the time interval from icv ropivacaine to the onset of first dysrhythmia, total dose of icv ropivacaine, and plasma concentration of ropivacaine

Group	n	t (min)	D (μg)	c (μg/ml)
1	20	16.9±3.2	833±159	0.23±0.09
2	18	17.8±4.3	863±221	0.25±0.11
3	20	17.9±2.8	885±137	0.19±0.05

Values are expressed as mean±SD. n_1: total case number; t: time interval from icv ropivacaine to onset of the first dysrhythmia; D: dose of icv ropivacaine; c: plasma concentration of ropivacaine

Ten minutes after icv ropivacaine and at the time of dysrhythmias developed, MAP increased significantly above baseline. During the icv administration of saline and ropivacaine, HR was different among each step, but such differences were not statistically significant (Table 4).

Table 4 Changes in MAP, HR, and the animal cases of dysrhythmias to be present in Group 1

Time	n_1	MAP (mmHg)	HR (beat/min)	n_2
Baseline	20	88±10.2	256±24	0
After icv saline	20	88±11.1	254±19	0
After icv ropivacaine				
5 min	20	92±12.1	262±25	0
10 min	20	117±15.6*	260±22	0
At the time for blood samples	20	123±13.7*	257±20	20

Values are expressed as mean±SD. * P<0.05 versus baseline. n_1: total case number; n_2: animal cases of dysrhythmias to be present; MAP: mean artery pressure; HR: heart rate

No animals had seizures in response to ropivacaine administration. There was no difference between CSF pressures at baseline ((2.9±0.75) cmH_2O) and at the point of maximal increase in MAP ((2.8±0.84) cmH_2O).

3.2 Group 2

Cardia dysrhythmias developed in all 20 rabbits in this group in response to icv ropivacaine (P=0.004) (Table 5). The average dose of ropivacaine that produced cardiac dysrhythmias was (863±221) μg ((0.173±0.044) ml). The time interval from icv ropivacaine to the onset of the first dysrhythmia was (17.8±4.3) min. Plasma ropivacaine concentrations were determined in all the animals in which cardiac dysrhythmias developed after icv ropivacaine. Plasma ropivacaine concentrations were undetectable in 2 of the 20 animals at the onset of dysrhythmias. In the 18 remaining animals, ropivacaine plasma concentrations averaged (0.25±0.11) μg/ml at the onset of dysrhythmias (Table 3).

Table 5 Changes in MAP, HR, and the animal cases of dysrhythmias to be present in Group 2

Time	n_1	MAP (mmHg)	HR (beat/min)	n_2
Baseline	20	87±13.2	247±21	0
At the time for blood samples	20	122±19.6*	255±24	20
After icv saline	20	125±20.3*	258±26	20
After icv midazolam	20	94±11.3	249±12	0
At the time of recurrence of dysrhythmias	16	119±10.8*	252±28	16
After icv midazolam secondly	16	90±12.5	242±19	0

Values are expressed as mean±SD. * P<0.05 versus baseline. n_1: total case number; n_2: animal cases of dysrhythmias to be present; MAP: mean artery pressure; HR: heart rate

In the animals with sustained dysrhythmias, icv saline had no effect on the dysrhythmias. However, icv midazolam terminated cardiac dysrhythmias within (4.8±0.9) min in all the animals (P=0.002); after having initially ceased, dysrhythmias recurred in 16 animals that received midazolam (Table 5). The average dysrhythmia-free interval after icv midazolam was (25±6.2) min. The time interval from the onset of dysrhythmia to the recurred dysrhythmia was (45±8.6) min. The 16 animals received a second dose

of midazolam, which terminated dysrhythmias in (3.3±0.6) min.

In response to ropivacaine infusion, blood pressure increased significantly above baseline. In animals that received midazolam after development of dysrhythmias, MAP returned to baseline values. In the 16 animals in which dysrhythmias recurred, MAP again increased significantly above baseline values. HR averaged (247±21) beats/min at baseline and did not significantly change after any treatment (Table 5).

No animals had seizures in response to ropivacaine administration. There was no difference between CSF pressures at baseline ((3.1±0.82) cmH_2O) and at the point of maximal increase in MAP ((2.9±0.73) cmH_2O).

3.3 Group 3

Dysrhythmias developed in all the animals in this group in response to icv ropivacaine (Table 6). The average dose of ropivacaine that produced dysrhythmias was (885±137) μg ((0.177±0.027) ml); the time interval from icv ropivacaine to the onset of the first dysrhythmia was (17.9±2.8) min; the plasma concentration of ropivacaine at the onset of dysrhythmias averaged (0.19±0.05) μg/ml (Table 3). In the animals with sustained dysrhythmias, the increase of the inspired isoflurane concentration from 0.75% to 1.50% had no effect on dysrhythmias in any of the animals (Table 6).

Table 6 Changes in MAP, HR, and the animal cases of dysrhythmias to be present in Group 3

Time	n_1	MAP (mmHg)	HR (beat/min)	n_2
Baseline	20	86±9.2	251±19	0
At the time for blood samples	20	119±17.8*	257±22	20
After isoflurane concentration increased				
5 min	20	95±9.2Δ	250±17	20
10 min	20	93±8.1Δ	260±25	20
15 min	20	92±8.3Δ	258±23	20

Values are expressed as mean±SD. * $P<0.05$ versus baseline; Δ $P<0.05$ versus the time for blood samples. n_1: total case number; n_2: animal cases of dysrhythmias to be present; MAP: mean artery pressure; HR: heart rate

In response to ropivacaine infusion, blood pressure increased significantly above baseline. After the inspired isoflurane concentration was increased from 0.75% to 1.50%, MAP decreased to baseline. The difference in HR was not statistically significant during each step (Table 6).

No seizures were noted in response to ropivacaine administration. There was no difference between CSF pressures at baseline ((2.8±0.51) cmH_2O) and at the point of maximal increase in MAP ((2.9±0.66) cmH_2O).

3.4 Group 4

Dysrhythmias developed in all animals in this group in response to iv phenylephrine infusion. Midazolam had no effect on dysrhythmias in any of the animals which suffered sustained dysrhythmias. In response to iv phenylephrine infusion, blood pressure increased significantly above baseline, and HR decreased significantly below baseline. In animals that received midazolam after dysrhythmias occurred, MAP and HR did not change significantly (Table 7).

Table 7 Changes in MAP, HR, and the animal cases of dysrhythmias to be present in Group 4

Time	n_1	MAP (mmHg)	HR (beat/min)	n_2
Baseline	20	87±10.1	255±22	0
After iv phenylephrine	20	111±13.7*	170±22*	20
After icv midazolam				
Instantly	20	125±25.2*	157±19*	20
5 min	20	119±13.1*	160±23*	20
10 min	20	117±10.3*	171±18*	20

Values are expressed as mean±SD. * $P<0.05$ versus baseline. n_1: total case number; n_2: animal cases of dysrhythmias to be present; MAP: mean artery pressure; HR: heart rate

Compared with the data from Groups 1, 2, and 3, there was no difference in baseline MAP ($P=0.24$) or maximum MAP ($P=0.21$). However, the onset of dysrhythmias in the icv ropivacaine-treated groups preceded the maximal increase in MAP by an average of (0.42±0.29) min, whereas in the iv phenylephrine-treated group, the onset of dysrhythmias occurred at an average of (5.12±1.67) min ($P<0.001$) after the maximal increase in MAP (Table 8).

There was no difference between CSF pressures at baseline ((2.6±0.36) cmH_2O) and at the point of maximal increase in MAP ((2.7±0.41) cmH_2O).

Table 8 Comparison of the time intervals from icv ropivacaine or iv phenylephrine to the onset of the first dysrhythmia and the onset of peak of MAP

Group	*n*	Time interval (min)	
		Dysrhythmias	Peak of MAP
1	20	16.9±3.2	16.8±4.1
2	20	17.6±3.9	18.3±4.5
3	20	17.9±2.8	18.1±3.8
4	20	6.1±1.7	1.9±0.3

Values are expressed as mean±SD

4 Discussion

Ropivacaine is a common local anesthetic agent of amide derivatives. Currently, most studies of ropivacaine cardiovascular toxicity have focused on the direct myocardial effect of ropivacaine as the mechanism producing cardiovascular toxicity. It is widely recognized that its cardiotoxic effect is mainly due to the negative inotropic effect and the abnormal conduction (Stehr *et al*., 2007; Udelsmann *et al*., 2009). However, there are some reports of CNS-related mechanisms being involved in the cardiovascular toxicity of local anesthetic (Stewart *et al*., 2003; Copeland *et al*., 2008). In Ladd *et al*. (2002)'s study, they performed an investigation that CNS and cardiotoxic effects were determined after bilateral carotid arterial infusions of levobupivacaine, bupivacaine, or ropivacaine in ewes. After pilot studies to validate the procedures were performed, equimolar doses (24–96 μmol, approximately 7.5–30.0 mg) were infused over 3 min using a crossover design. Their results showed that blood drug concentrations in the superior sagittal sinus were 5–10 times greater than those concurrently in the aorta, confirming highly selective CNS delivery with minimal systemic recirculation. Dose-dependent CNS excitatory behavior and electroencephalogram (EEG) changes, with increased mean arterial blood pressure, HR, cardiac output, and myocardial contractility were found consistent with sympathetic nervous system stimulation.

Our results demonstrate that icv ropivacaine produces dysrhythmias and increases artery blood pressure, which are associated with iv ropivacaine administration (Dony *et al*., 2000; Guinet *et al*., 2009). Ropivacaine has been shown to induce neurotoxicity following overdose or accidental intravenous injection (Lin *et al*., 2007; Rodolà *et al*., 2007). These results showed that ropivacaine can penetrate the blood-brain barrier and then enter brain tissue. In Guilhaumou *et al*. (2010)'s study, two groups with six rats each were given a single subcutaneous ropivacaine injection, and then the hyperthermia-induced animals were placed in a water bath to obtain a stable mean core temperature of 39.7 °C; the other two groups with six rats each were sacrificed 30 min after ropivacaine injection to determine the ropivacaine concentrations in serum and tissues (brain and heart). Their results revealed a significant increase of the total apparent clearance ((15.1±0.8) ml/min vs. (13.4±0.134) ml/min) and apparent volume of distribution ((2.19±0.27) L vs. (1.57±0.73) L), and a significant decrease in exposure ((488±50.6) L vs. (572±110) L) in induced-hyperthermia group. They observed a significant increase in brain ropivacaine concentration in hyperthermic rats ((8.39±8.42) μg/g vs. (3.48±3.26) μg/g), but no significant difference in cardiac ropivacaine concentrations between the two groups ((5.38±4.83) μg/g vs. (3.73±2.44) μg/g). Their results suggest a higher tissular distribution of ropivacaine and an increase in blood-brain barrier permeability during hyperthermia. In our study, plasma concentrations of ropivacaine at the onset of dysrhythmias averaged (0.23±0.05) μg/ml in the animals from Groups 1, 2, and 3 in which dysrhythmias developed after icv ropivacaine. These concentrations are well below the concentrations ((12.36±1.06) μg/ml) found in rabbits given iv ropivacaine to produce dysrhythmias (Velly *et al.*, 2006; Moore, 2009). Therefore, direct myocardial toxicity of ropivacaine is an unlikely explanation for the dysrhythmias.

The absence of significant change in CSF pressure from baseline to each time of drug administration indicates that dysrhythmias were not the result of increased CSF pressure.

Our data showed that icv midazolam terminated dysrhythmias produced by icv ropivacaine in the animals, and that increasing anesthetic depth with isoflurane had no effect on dysrhythmias produced by icv ropivacaine. These results suggest that the effects of midazolam are specific to its ability to potentiate GABA (Asl *et al.*, 2008; Igarashi *et al.*, 2009) and are not the result of generalized CNS depression. We also

found that icv midazolam decreased artery blood pressure produced by icv ropivacaine, and that increasing anesthetic depth with isoflurane significantly decreased the artery blood pressure produced by icv ropivacaine in the animals, which indicated that the decrease in artery blood pressure was not simply the result of icv midazolam. Several studies (Greenblatt *et al.*, 1992; Kanaya *et al.*, 1998) have suggested that isoflurane modifies responses to endothelium-dependent vasodilators and peripheral vascular resistance, which leads to the decrease of artery blood pressure. Experiments (Wiktorowska *et al.*, 1999; Novellas *et al.*, 2007) have shown that iv midazolam caused a gradual fall of artery blood pressure. In Ahmad *et al.* (2000)'s study, two groups of piglets (1–3 d old, 1.0–1.5 kg) received either (1) a loading dose of 300 μg/kg of midazolam over 15 min, followed by a continuous intravenous infusion of 100 μg/(kg·h) (n=6), or (2) equivalent volume bolus and intravenous infusions of 0.05 g/ml dextrose (control, n=8). Systemic and cerebral venous midazolam concentrations were measured at 0.5, 1, 2, 3, 4, 5, and 6 h. Midazolam infusion did not affect systemic hemodynamics or blood gases. In contrast, midazolam infusion significantly reduced sagittal sinus vein blood pressure, PaO_2, oxygen saturation, and oxygen content. Cerebral fractional oxygen extraction increased and was positively correlated with cerebral fractional midazolam extraction. So they concluded that midazolam infusion preceded by a high bolus dose in newborn piglets alters sagittal sinus vein blood pressure and cerebral fractional oxygen extraction. These changes may reflect decreased brain perfusion and metabolism during midazolam infusion. Nishiyama *et al*. (2003) showed that epidurally administered midazolam enters the CSF, but the concentration was only 3% of that in the systemic circulation.

The recurrence of dysrhythmias, after they initially ceased in response to icv midazolam, likely resulted from a waning of the effect of midazolam and the re-emergence of the unopposed effect of ropivacaine. The recurrence of dysrhythmias showed that the initial terminating of dysrhythmias after icv midazolam did not result from a spontaneous remission. In Group 1, the duration of ropivacaine-induced dysrhythmias lasted (69±11) min; in Group 2, the average dysrhythmia-free interval after icv midazolam was (25±6.2) min. The time interval from the onset of dysrhythmia to the dysrhythmia recurring after the first icv midazolam was (45±8.6) min, which may be the reason for the recurrence of dysrhythmias after having initially ceased in response to icv midazolam.

Rising plasma levels of ropivacaine can lead to a progressive spectrum of neurological and cardiac effects. Seizure activity may herald the onset of myocardial depression and ventricular arrhythmias that are often refractory to treatment (Kimura *et al*., 2007; Copeland *et al*., 2008; Wan *et al*., 2010). However, in our study, no animals suffered seizures in response to icv ropivacaine administration. This observation suggests that the termination of seizures may be caused by general anesthesia. Isaeva (2008), examined the effect of isoflurane on seizure-like activity at hippocampal CA3 pyramidal region of immature rats in vivo, and found that isoflurane at clinically-relevant concentrations effectively halted hippocampal seizures.

To determine whether the dysrhythmias produced by icv ropivacaine were simply the result of ropivacaine-induced hypertension, we studied a group of animals in which dysrhythmias were produced by iv phenylephrine infusion. We found that hypertension produced by iv phenylephrine did induce dysrhythmias, but that the time course of those dysrhythmias was significantly different from that produced by ropivacaine. The icv ropivacaine-induced dysrhythmias occurred at an average of (0.42±0.29) min before the maximal increase in MAP, whereas iv phenylephrine-induced dysrhythmias followed the maximal increase in MAP by an average of (5.12±1.67) min. These results demonstrate that icv ropivacaine-induced dysrhythmias are coincident with the ropivacaine-induced increase in MAP, but are not a result of that increase. Therefore, midazolam had no effect on dysrhythmias produced by iv phenylephrine in these animals. In contrast, icv midazolam terminated dysrhythmias produced by icv ropivacaine.

We suggest that ropivacaine cardiotoxicity may be divided into an early stimulatory component, which we believe to be CNS-mediated, and a later depressant component that is the result of direct myocardial effects of the drug (Tsibiribi *et al*., 2006). Whether or not preventing or treating this early

component of ropivacaine cardiotoxicity can decrease the mortality of accidental iv ropivacaine injections remains to be determined. However, because refractory ventricular dysrhythmias are such a prominent part of human ropivacaine toxicity, it is important to investigate the possibility that early dysrhythmias caused by ropivacaine actions in the CNS may contribute to the late cardiac mortality of ropivacaine.

We have shown that the administration of ropivacaine into the cerebral ventricles of rabbits results in CNS-mediated cardiac dysrhythmias and that these CNS-mediated dysrhythmias could be terminated by CNS administration of midazolam that enhances GABAergic activity. However, whether or not the CNS-mediated cardiotoxicity that occurs after direct CNS administration of ropivacaine contributes to the cardiotoxicity of ropivacaine following intravenous administration remains to be determined.

In conclusion, we confirm that ropivacaine administered directly into the CNS is capable of producing cardiac dysrhythmias. Further, we have shown that midazolam terminated dysrhythmias presumably by potentiation of GABA receptor activity. Our results suggest that ropivacaine produces some of its cardiotoxicity by a CNS mechanism. Further efforts to study the toxicity of ropivacaine may enhance our understanding of not only the direct cardiotoxicity of the drug, but also the CNS effects of ropivacaine.

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