Effect of Intracerebroventricular Picrotoxin and Muscimol on Intravenous Bupivacaine Toxicity

Evidence Supporting Central Nervous System Involvement in Bupivacaine Cardiovascular Toxicity

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Background: The authors have previously shown that administration of bupivacaine 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 a drug (midazolam) that enhances GABA-ergic activity. The goal of the current investigation was to determine whether the CNS-mediated cardiotoxicity that occurs after direct CNS administration of bupivacaine contributes to bupivacaine’s cardiovascular toxicity following intravenous administration.

Methods: Three groups of rabbits were pretreated by intracerebroventricular administration of mock CSF (control), muscimol (a GABA agonist), or picrotoxin (a GABA chloride channel blocker). A fourth group received intravenous hexamethonium as a pretreatment. After pretreatment, all groups received intravenous bupivacaine at 1.5 mg·kg⁻¹·min⁻¹ and the bupivacaine dose and plasma concentration at the onset of dysrhythmias and cardiovascular collapse were determined. It was hypothesized that pretreatment with muscimol and hexamethonium would increase the threshold for cardiac dysrhythmias, while pretreatment with picrotoxin would decrease the threshold for dysrhythmias.

Results: The bupivacaine plasma concentration required to produce cardiac dysrhythmias was significantly greater in the muscimol and hexamethonium groups compared to control. The bupivacaine plasma concentration at the onset of dysrhythmias was not different from control in the picrotoxin group. The bupivacaine plasma concentration that produced cardiovascular collapse did not differ from control in any of the groups.

Conclusions: It was concluded that the data generally support the hypothesis that bupivacaine-mediated cardiac dysrhythmias are, at least in part, mediated by CNS actions of the drug. (Key words: Anesthetics, local: bupivacaine. Gamma-aminobutyric acid. Muscimol. Picrotoxin. Toxicity.)

BUPIVACAINE, a potent long-acting amide local anesthetic, has been associated with sudden cardiovascular collapse and fatal ventricular dysrhythmias after intravascular injection in humans and laboratory animals.1-4 Numerous animal studies aimed at elucidating the mechanism of bupivacaine’s cardiovascular toxicity have focused on the direct myocardial effects of the drug as the principal mechanism of toxicity. These studies have clearly shown that bupivacaine can produce cardiotoxic effects, including impaired contractility and conduction, by direct actions on the myocardium.5-10

However, several studies have demonstrated that the direct application of local anesthetics, especially bupivacaine, into the central nervous system (CNS) can also produce cardiac dysrhythmias and sudden death.11-13 These studies have led to speculation that the cardiovascular toxicity of bupivacaine is mediated, at least in part, by drug actions within the CNS. Although results from these studies are provocative, direct CNS application of bupivacaine does not reproduce the clinical situation of accidental intravascular injection. Consequently, it is difficult to conclude that intravenous administration of bupivacaine produces cardiovascular toxicity by a CNS mechanism.

In a previous study, we demonstrated that direct CNS administration of bupivacaine by ventriculocisternostomy perfusion (VCP) produced cardiac dysrhythmias and hypertension.13 In addition, we showed that intracerebroventricular administration of midazolam, a drug that enhances gamma-aminobutyric acid (GABA) activity, terminated both the cardiac dysrhythmias and hypertension. From these and other data, we speculate that the dysrhythmias and hypertension caused by in-
tracerebroventricular bupivacaine result from increased brainstem sympathetic nervous system (SNS) outflow. The increased SNS outflow could result from bupivacaine-mediated blockade of the GABA-ergic neurons that are known to tonically inhibit brainstem sympathetic outflow. Midazolam was effective in terminating the dysrhythmias and hypertension because it restored the lost GABA-ergic inhibition. Although this study suggests a mechanism by which bupivacaine produces cardiovascular toxicity when administered directly into the CNS, it does not address the question of whether or not the CNS plays a role in bupivacaine’s cardiovascular toxicity after accidental intravascular injection.

The goal of the current investigation was to determine whether the CNS-mediated cardiotoxicity that occurs after intracerebroventricular administration of bupivacaine contributes to bupivacaine’s cardiovascular toxicity after intravenous administration. To address this question, we altered brainstem GABA-ergic activity by pretreating rabbits by intracerebroventricular administration of muscimol, a GABA-receptor agonist; picrotoxin, a GABA chloride channel blocker; or mock cerebrospinal fluid (CSF) (control) before an intravenous infusion of bupivacaine. We reasoned that, if bupivacaine redistribution into the brainstem during intravenous administration contributes to bupivacaine’s cardiovascular toxicity by blocking brainstem GABA neurons, then the animals pretreated with muscimol should develop cardiovascular toxicity at a higher bupivacaine plasma concentration compared with the animals pretreated with mock CSF, while animals pretreated with picrotoxin may develop cardiovascular toxicity at a lower plasma concentration.

Materials and Methods

Animal use was approved by the University of Washington Animal Care Committee. American Association for Laboratory Animal Care guidelines were followed throughout the study.

Surgical Preparation

Thirty-seven New Zealand white rabbits, of both sexes, weighing 3.3–4.7 kg, were anesthetized with halothane (1–2%) and N2O (60%) in oxygen. After tracheal intubation, the lungs were ventilated with a Harvard pump. Expired CO2 was continuously monitored (Capnogard model 1250, Novametrix, Wallingford, CT) and arterial blood gases were measured to verify that expired CO2 measurements accurately reflected PaCO2. Ventilation was adjusted, as indicated by end-tidal CO2 measurements, to maintain normocapnia. The right femoral artery was cannulated for blood pressure monitoring and blood sampling. The right femoral vein was cannulated for venous access.

Twenty-nine rabbits were surgically prepared for VCP. The head was secured with blunt prongs in a stereotactic frame and the skull 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 posterior to the coronal suture. A 20-G needle was inserted through this hole into the left lateral ventricle. A T-piece connected to this needle allowed simultaneous perfusion of the lateral ventricle and measurement of ventricular CSF pressure. The posterior neck muscles were dissected and a 20-G cannula was inserted between the first cervical vertebra and the base of the cranium and into the cisterna magna. Continuous measurement of ventricular and cisternal CSF pressure allowed selection of a rate of intraventricular drug administration that did not increase CSF pressure.

Ventriculocisternal perfusion was established by perfusing mock CSF (pH = 7.38–7.42; mosm = 295–300) through the ventricular cannula and was considered successful if CSF pressure did not increase to greater than preperfusion values and mock CSF flowed from the cisternal cannula. Ventriculocisternal perfusion was controlled by a syringe pump at a rate of 0.057 ml/min.

Needle electrodes were inserted at both shoulders and both thighs to monitor the electrocardiogram (ECG). The right hemispheric electroencephalogram (EEG) was monitored (Lifescan™ Brain Activity Monitoring System, Diatek Medical™ Technology Incorporated, San Diego, CA) using gold cup electrodes placed over the right frontal cortex and the parieto-occipital cortex. Blood pressure, heart rate, CSF pressure, ECG, and end-tidal CO2 were continuously recorded on a strip chart recorder.

After completing the surgical preparation, halothane was decreased to 0.25% inspired concentration, N2O was increased to 70% and the animals were paralyzed by continuous intravenous infusion of pancuronium bromide (40–80 μg/h). The EEG was continuously monitored as an indicator of anesthetic depth and to provide evidence of seizure activity. The predominance of low-frequency activity and the near absence of high-frequency activity indicated that the animals were adequately anesthetized after the decrease in anesthetic

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depth. After the change in anesthetic, we allowed at least 20 min to elapse before beginning the experiments.

One group of animals (n = 7) was pretreated with intracerebroventricular mock cerebral spinal fluid (100 μl/min) before intravenous bupivacaine infusion; this group served as the control group. A second group of animals (n = 11) was pretreated by intracerebroventricular administration of 10 μg of the GABA-agonist muscimol dissolved in mock CSF (1 μg/min; 100 μl/min). A third group of animals (n = 11) was pretreated by intracerebroventricular administration of 10 μg of the GABA chloride channel blocker picrotoxin (1 μg/min; 100 μl/min). This dose of picrotoxin was chosen because it was the largest dose that itself did not produce cardiac dysrhythmias. We also studied a fourth group of animals (n = 8) that was not surgically prepared for VCP. This group was pretreated with intravenous hexamethionum. We studied this group of animals because our earlier study demonstrated that intravenous hexamethionum also terminated dysrhythmias and hypertension caused by intracerebroventricular bupivacaine. The animals in the hexamethionum group did not have ventricular or cisternal cannulae placed, but were in all other ways treated exactly as were the VCP animals. Hexamethionum (200 μg/ml) was administered by continuous intravenous infusion for 10 min at a rate sufficient to decrease MAP by 5–10 mmHg.

Immediately after pretreatment, all animals received an intravenous infusion of bupivacaine at 1.5 mg·kg⁻¹·min⁻¹ by positive pressure pump until cardiovascular collapse (defined as mean arterial pressure = 25 mmHg). During bupivacaine infusion, the processed EEG was continuously monitored for seizure activity. Arterial blood samples (4 ml) were drawn at the onset of cardiac dysrhythmias and at cardiovascular collapse to measure bupivacaine plasma concentrations. Dysrhythmias were considered to be any abnormal ventricular rhythm (e.g., ectopic beats occurring more than five times per minute, bigeminy, trigeminy, or ventricular tachycardia). The plasma was separated from arterial blood samples and stored frozen at −40°C until analyzed.

Bupivacaine Analysis

Plasma samples were assayed for total bupivacaine by a modification of the method of Mather and Tucker. Briefly, this method involves addition of an internal standard (W38088, Astra Pharmaceuticals, Westborough, MA), extraction of the drug from plasma, separation by gas chromatography, and detection with a nitrogen-phosphorus detector. The bupivacaine assay has a sensitivity limit of 1.1 ng/ml base and a coefficient of variation of 4.7% at 50 ng/ml.

Statistical Analysis

Differences between experimental groups and the control group for blood pressure and heart rate response to bupivacaine infusion were analyzed by ANOVA for repeated measures, and Dunnet’s test was used for post hoc testing. Within-group differences between baseline blood pressure and heart rate and blood pressure and heart rate after pretreatment were assessed by ANOVA for repeated measures, and Student’s paired t test was used for post hoc comparison. Differences between groups in the dose to first dysrhythmia, dose to cardiovascular collapse, plasma concentration at first dysrhythmia, and plasma concentration at cardiovascular collapse were analyzed by ANOVA, and Dunnet’s test was used for post hoc comparison with the control group. Differences were considered statistically significant at P < 0.05 to determine whether there was a relationship between cardiovascular variables and the threshold for dysrhythmias, we compared MAP and heart rate to the plasma bupivacaine concentration at the onset of dysrhythmias by least-squares linear regression and calculation of the determination coefficient (R²).

Results

At baseline (the time at which pretreatment with mock CSF, muscimol, picrotoxin, or hexamethionum was begun), the treatment groups did not differ from the control group with respect to weight, arterial blood gases, heart rate, or MAP (table 1, figs. 1 and 2).

The plasma concentration of bupivacaine that produced cardiac dysrhythmias in the muscimol and hexamethionum groups was significantly greater than in the control group (table 2). The plasma concentration of
bupivacaine that produced cardiac dysrhythmias in the picrotoxin group did not differ from control (table 2). The dose of bupivacaine that produced cardiac dysrhythmias was not different from control in any of the groups. In all groups, cardiac dysrhythmias initially consisted of premature ventricular contractions (PVC). The dysrhythmias progressed to frequent multifocal PVC, bigeminy, trigeminy, or brief runs of ventricular tachycardia. No animal developed ventricular fibrillation and no animal died of ventricular dysrhythmias.

The plasma concentration of bupivacaine at cardiovascular collapse was not significantly different from control in any of the groups (table 2). However, the dose of bupivacaine required to produce cardiovascular collapse was significantly less than control in the muscimol and hexamethonium groups, while the dose did not differ from control in the picrotoxin group (table 2). In all animals, cardiovascular collapse resulted from electromechanical dissociation (EMD).

Heart rate data are presented for the first 3.5 min of bupivacaine infusion (fig. 1). Beyond 3.5 min, some animals developed such severe dysrhythmias that it was not possible to discern their underlying heart rate. Pretreatment with mock CSF, hexamethonium, and picrotoxin had no significant effect on heart rate, while muscimol pretreatment significantly reduced heart rate. During bupivacaine infusion, heart rate in the muscimol group remained significantly below the control group until 2.0 min, after which time there was no significant difference between the two groups. Heart rate in the picrotoxin and hexamethonium groups did not differ from the control group at any time during the first 3.5 min of bupivacaine infusion.

Blood pressure data are presented for the first 4.5 min of bupivacaine infusion (fig. 2). Although there are data for most animals beyond 4.5 min, some animals developed cardiovascular collapse between 4.5 and 5 min, resulting in incomplete data sets for statistical analysis. Pretreatment with intracerebroventricular mock CSF had no statistically significant effect on mean arterial pressure in the control group (fig. 2). In contrast, muscimol and hexamethonium pretreatment produced a significant decrease in MAP, while picrotoxin pretreatment produced a nonsignificant increase in MAP (fig. 2). At the onset of intravenous bupivacaine infusion, MAP in the muscimol and hexamethonium groups was significantly below the control group and remained so until shortly before cardiovascular collapse. In the picrotoxin group, MAP did not differ significantly from control.

To determine whether or not differences in threshold for cardiac dysrhythmias were related to differences in MAP and/or heart rate between the groups, we plotted the MAP (fig. 3) and the heart rate (fig. 4) at which dysrhythmias occurred against the plasma bupivacaine concentration at the onset of dysrhythmias. In so doing, we hoped to determine whether there was a significant correlation between heart rate or MAP and the plasma concentration of bupivacaine required to produce dysrhythmias. The determination coefficients (R²) for plots.
of heart rate versus plasma bupivacaine concentration at the onset of dysrhythmias ranged from 0.018 (muscimol) to 0.097 (picrotoxin). The values for the slopes relating heart rate to bupivacaine plasma concentration ranged from -0.0009 to -0.0014. The determination coefficients ($R^2$) for plots of MAP versus plasma bupivacaine concentration at the onset of dysrhythmias ranged from 0.000 (muscimol) to 0.515 (control). The values for the slope relating MAP to bupivacaine plasma concentration ranged from -0.13 to +0.31.

Ventriculocisternal perfusion with mock CSF, muscimol, or picrotoxin did not alter CSF pressure, indicating that observed changes in heart rate and blood pressure were not the result of changes in CSF pressure.

No animal developed EEG evidence of seizures in response to bupivacaine infusion, although some animals developed a burst suppression pattern on the EEG. At cardiovascular collapse, the EEG fell silent in all animals, presumably because of inadequate perfusion, not because of CNS effects of bupivacaine.

**Discussion**

Local anesthetics administered intravenously have been shown to produce cardiovascular stimulation by a CNS mechanism.\textsuperscript{15} In our previous study, we hypothesized that this CNS-mediated cardiovascular stimulation produced by local anesthetics may play a role in bupivacaine cardiovascular toxicity. To test this hypothesis, we administered bupivacaine by VCP and demonstrated that direct CNS administration of bupivacaine can produce cardiotoxic effects by actions within the CNS. In addition, we demonstrated that the CNS-mediated cardiotoxic effects of bupivacaine can be terminated by a drug (midazolam) that enhances GABA-ergic activity or by a drug that blocks autonomic ganglia (hexamethonium). These findings were consistent with the fact that the GABA-ergic system is the principal inhibitor of sympathetic outflow from the brainstem.\textsuperscript{16-18} Based on these data, we speculated that bupivacaine blocked the brainstem GABA-ergic neurons that tonically inhibit sympathetic outflow. The result of lost GABA-ergic inhibition was an increase in sympathetic outflow that, in turn, produced the observed dysrhythmias and hypertension. We speculated that the reason midazolam terminated dysrhythmias and hypertension was because it restored the lost GABA-ergic inhibition. Hexamethonium terminated dysrhythmias because it blocked the increased sympathetic outflow at the level of the autonomic ganglia.

Our previous study provided a working model to explain the dysrhythmias and hypertension that occur after direct CNS administration of local anesthetics, particularly bupivacaine. However, the important question is whether the CNS-mediated cardiotoxic effects of bupivacaine play a role in bupivacaine-induced cardiovascular toxicity after intravenous administration. In the current investigation, we hypothesized that intravenous bupivacaine does produce cardiotoxic effects, at least in part, by the same mechanism shown to operate after direct CNS administration. To test this hypothesis, we pretreated rabbits with either muscimol or picrotoxin before intravenous bupivacaine infusion. Because enhancement of GABA-ergic activity was previously shown to terminate the CNS-mediated cardiovascular effects of intracerebroventricular bupivacaine, we expected that the muscimol group would be less sensitive to the toxic effect of intravenous bupivacaine and the picrotoxin group would be more sensitive. Our finding that muscimol pretreatment increased the threshold for bupivacaine-induced cardiac dysrhythmias is, therefore, consistent with our hypothesis. This

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finding is also consistent with the findings of Bernards et al., who showed that intravenous benzodiazepine pretreatment raised the threshold for intravenous bupivacaine-induced dysrhythmias in pigs.\(^4\)

In contrast, we found that picROTOXIN pretreatment did not decrease the threshold for cardiac dysrhythmias as hypothesized. The reason for this is unclear. It is possible that a larger dose would have demonstrated the expected effect, but, in pilot studies, larger doses were shown to produce spontaneous dysrhythmias in some animals. The ability of intracerebroventricular picROTOXIN to produce cardiac dysrhythmias is consistent with the work of others.\(^19\) Because picROTOXIN-induced dysrhythmias would have invalidated the study, we chose a conservative picROTOXIN dose. The fact that MAP and heart rate in the picROTOXIN group were not significantly greater than the control group during bupivacaine infusion supports the idea that the dose was inadequate to produce significant GABA chloride channel blockade. Unfortunately, doses that produce sig-
Fig. 4. Plots of mean heart rate versus plasma concentration of bupivacaine at the onset of dysrhythmias for each group. Least-square linear regression equations and determination coefficients (R^2) can be found at the bottom of each plot.

Significantly greater MAP and heart rate also produce dysrhythmias.

Given that muscimol and hexamethonium increased the threshold for cardiac dysrhythmias, but did not alter the threshold for cardiovascular collapse, it is important to question whether the increased dysrhythmia threshold is clinically significant. To answer this question, it is important to realize that, in both human clinical experience and laboratory animal studies, death from intravenous bupivacaine occurs from either ventricular dysrhythmias or “pump failure” (EMD). Which of these mechanisms produces death is subject to both species and individual variability. For example, Bernards et al. have given fatal doses of bupivacaine to 50 pigs, all of which died of EMD.4,20 Kasten and Martin gave fatal doses of bupivacaine to both dogs and sheep; all of the dogs died of EMD, while the sheep died of ventricular fibrillation.21 In contrast, Rosen et al. reported EMD as the most common cause of death in sheep given toxic doses of bupivacaine.22 Albright reported six anecdotal
cases of sudden cardiovascular collapse after intravenous injection of bupivacaine or etidocaine in which patients died of both ventricular dysrhythmias and EMD. It is clear that both ventricular dysrhythmias and EMD can cause death after accidental intravenous injection of bupivacaine; however, it is unclear why dysrhythmias predominate in some species or individuals and EMD predominates in others. We speculate that, in those individuals in which ventricular dysrhythmias are the cause of death, increasing the threshold for dysrhythmias may also raise the threshold for cardiovascular collapse.

A criticism of our study would be that pretreatment with muscimol and hexamethonium altered the MAP and heart rate of the animals and that the altered hemodynamics are responsible for the raised dysrhythmia threshold, not blockade of CNS-mediated effects of bupivacaine. Unfortunately, hemodynamic changes produced by the pretreatment regimens are unavoidable, because all of these drugs have well-defined effects on the autonomic nervous system. However, we would point out that the hexamethonium group showed a decrease in MAP that was similar to that in the muscimol group, but had a heart rate that did not differ from control. Because both the muscimol and hexamethonium groups demonstrated an increased threshold for bupivacaine-induced dysrhythmias, heart rate would seem not to have been an important determinant of dysrhythmia threshold. In addition, as demonstrated in figure 3, there was a poor correlation between heart rate and bupivacaine plasma concentration at the onset of dysrhythmias. We conclude from these data that heart rate was not an important determinant of dysrhythmia threshold. Figure 4 demonstrates a better correlation between MAP and bupivacaine plasma concentration at the onset of dysrhythmias in the control and hexamethonium groups, but not in the muscimol or picROTOXIN groups. However, the slopes relating MAP and bupivacaine plasma concentration are both positive and negative, and the values for the slopes differ several-fold among the groups. We conclude from these data that MAP had little effect on the threshold for cardiac dysrhythmias.

In addition to the hemodynamic changes induced by pretreatment with hexamethonium and muscimol, both drugs appear to have altered bupivacaine pharmacokinetics as well. With both drugs, a given dose of bupivacaine resulted in a higher bupivacaine plasma concentration than was achieved in the control group. The most likely explanation for this pharmacokinetic difference is that muscimol and hexamethonium pretreatment decreases bupivacaine's volume of distribution. However, it is the plasma concentration of bupivacaine, not the dose, that determines its pharmacodynamic effects. Therefore, we do not believe the altered kinetics explain the observed differences between the groups.

Clarkson and Hondegem have demonstrated, in isolated guinea pig ventricular muscle, that the degree of myocardial sodium channel blockade by bupivacaine increases as heart rate increases. Increasing sodium channel blockade, in turn, results in greater depression of myocardial conduction. More recently, de La Coussaye et al. have demonstrated rate-dependent conduction block and reentrant dysrhythmias in an in vitro rabbit heart model. Both of these studies have led to speculation that bupivacaine-induced dysrhythmias result from conduction block and subsequent reentry. However, to our knowledge, no study has ever investigated the effect of heart rate on dysrhythmia threshold in an intact animal. Our finding that heart rate did not correlate well with the threshold for cardiac dysrhythmias in any of our four groups is not consistent with the findings of Clarkson and Hondegem or de La Coussaye et al. The reason for this apparent inconsistency is unclear, but may simply reflect the fact that the intact animal is a much more complex system than the isolated heart or muscle strip. Another possible explanation is that, in our intact rabbit model, dysrhythmias are caused by a mechanism other than conduction block and reentry, e.g., early or late afterdepolarizations. Whatever the explanation, the lack of a correlation between heart rate and dysrhythmia threshold does raise the question of whether the greater rate-dependent block seen with bupivacaine compared with lidocaine is responsible for the seemingly greater in vitro cardiototoxicity of bupivacaine compared with other local anesthetics.

In summary, we found that enhancing brainstem GABA-ergic activity by intracerebroventricular administration of muscimol raised the threshold for intravenous bupivacaine-induced cardiac dysrhythmias. This finding is consistent with our hypothesis that bupivacaine produces cardiac dysrhythmias, in part, by blocking the GABA-ergic neurons that normally inhibit central sympathetic outflow. Our finding that peripheral sympathetic blockade with hexamethonium also increased the threshold for intravenous bupivacaine-induced dysrhythmias is also consistent with our hypothesis. Because hexamethonium is a general autonomic ganglion blocker, it is possible that parasym-
Pathetic blockade also contributed to the ability of hexamethonium to raise the threshold for bupivacaine-induced dysrhythmias.

In contrast, intracerebroventricular picrotoxin pre-treatment did not decrease the threshold for bupivacaine-induced dysrhythmias as expected. We speculate that the lack of an effect by picrotoxin may reflect the fact that the dose of picrotoxin was inadequate to produce significant chloride channel blockade. Although the data are not conclusive, we believe they support the hypothesis that intravenous bupivacaine produces cardiac dysrhythmias, in part, by blocking GABA-ergic activity in the brainstem.

References