Bupivacaine Toxicity in Young Pigs Is Age-Dependent and Is Affected by Volatile Anesthetics

J. Michael Badgwell, M.D.,* James E. Heavner, Ph.D., D.V.M.,† Juha Kyutta, M.D.‡

The influence of age and volatile anesthetic agents on plasma concentrations and toxic effects of bupivacaine were studied in 2-day-old, 2-week-old, and 2-month-old pigs. Bupivacaine was infused at a constant rate while the pigs' ECGs and EEGs were recorded. Six pigs in each age group were lightly anesthetized with 70% N\textsubscript{2}O/30% O\textsubscript{2} during the bupivacaine infusion, and twelve 2-day-old pigs were anesthetized with 70% N\textsubscript{2}O/30% O\textsubscript{2} plus ether. 0.5 × MAC halothane or isoflurane. Two-day-old pigs were more resistant than older pigs to the toxic effects of bupivacaine despite higher plasma concentrations at all sample times. All pigs given N\textsubscript{2}O alone or N\textsubscript{2}O plus halothane had ventricular dysrhythmias, but only one pig in the N\textsubscript{2}O plus isoflurane group had a ventricular dysrhythmia. Threshold doses of bupivacaine for dysrhythmias in the N\textsubscript{2}O alone and N\textsubscript{2}O plus halothane groups did not differ. Seizures occurred in all pigs in the N\textsubscript{2}O alone group, in none of the N\textsubscript{2}O plus halothane group, and in two of the N\textsubscript{2}O plus isoflurane group. The doses required to depress cardiac index and cause asystole were less in the groups receiving halothane and isoflurane. It was concluded that N\textsubscript{2}O plus halothane and N\textsubscript{2}O plus isoflurane increase the lethality of bupivacaine while preventing early warning signs of toxicity. (Key words: Age factors, toxicity: central nervous system; heart. Anesthesia: pediatric. Anesthesics, local: bupivacaine, toxic effects. Anesthetics, volatile: halothane; isoflurane.)

THE USE OF REGIONAL anesthesia for reduction of intraoperative general anesthetic requirements and for postoperative pain relief has become increasingly popular for pediatric patients. This practice is considered extremely safe, and reported complications are rare. However, intravascular injection of local anesthetic agents may occur and may cause serious sequelae, including seizures, dysrhythmias, and cardiac arrest.

Toxic doses of local anesthetics in infants and children are unknown, but they have been extrapolated from data in adults. The margin of safety, however, for local anesthetic toxicity may be less in newborns and infants who have low plasma concentrations of α\textsubscript{1}-acid glycoprotein.

Methods

After approval from the Animal Care and Use Committee, we studied 30 farm-bred female pigs aged 2 days (n = 18), 2 weeks (n = 6), and 2 months (n = 6). Based on their ages at onset of puberty (7 months for pigs and 13 yrs for menarche in human females), a 2-day-old pig would be analogous to a 6-week-old infant, a 2-week-old pig would be analogous to a 10-month-old infant, and a 2-month-old pig would be analogous to a 3½-year-old child. In all pigs, anesthesia was induced using 70% N\textsubscript{2}O/30% O\textsubscript{2} and halothane. After tracheal intubation with a cuffed tube, ventilation was controlled with a Siemens-Elema 900D Ventilator. End-tidal P\textsubscript{CO\textsubscript{2}}, sampled from the distal tip of the endotracheal tube, was measured using infrared analysis and was maintained between 32 and 36 mmHg. The ECG and fronto-occipital EEG were continuously recorded on a strip chart. Body temperature was measured rectally and maintained with heating blankets in the range of 36° C–37°C.

During surgical preparation (insertion of catheters described below), anesthesia was maintained with 70% N\textsubscript{2}O/30% O\textsubscript{2} and either halothane (1.0–1.5% inspired, n = 24) or isoflurane (01.5–2.0% inspired, n = 6), depending on which anesthetic agent would be used for maintenance anesthesia. Halothane was used in pigs who were to receive N\textsubscript{2}O and O\textsubscript{2} alone (see below). Catheters were inserted into the 1) femoral artery for continuous blood pressure measurement, 2) inferior vena cava (via the femoral vein)
for drug and fluid administration and, 3) contralateral femoral vein for blood sampling. Venous blood was sampled instead of arterial blood to avoid either the need for an additional arterial catheter (which might reduce the central compartment) or interference with the arterial blood pressure recording during blood sampling. It has been demonstrated that under conditions similar to those in this study, central venous and arterial concentrations of bupivacaine are essentially equal.10

After catheterization, the pigs were given lactated Ringer’s solution with 5% dextrose (25 ml·kg−1) and pancuronium (0.15 mg·kg−1) iv. Neuromuscular blockade was maintained as needed with additional pancuronium. Some cardiovascular disturbances occurred following pancuronium injection: most notably, a transient increase in heart rate and blood pressure followed in some cases by a transient decrease in blood pressure. Therefore, we did not give pancuronium within less than 15 min before bupivacaine infusion was started. Usually the level of neuromuscular blockade was such that twitching of facial muscles accompanied EEG evidence of seizure activity.

The study was then divided into parts A and B. In part A, we studied the effect of age on plasma concentrations and toxicity of bupivacaine. In 2-day-old, 2-week-old, and 2-month-old pigs (n = 6 in each group), anesthesia was maintained using 70% N2O/30% O2 (N2O alone). In part B, we studied the effect of volatile anesthetic agents on the plasma concentrations and toxicity of bupivacaine. In two groups of 2-day-old pigs (n = 6 in each group), anesthesia was maintained using 70% N2O/30% O2 and either 0.5-MAC end-tidal halothane or isoflurane. The MAC in newborn pigs is 0.95 ± 0.02% for halothane and 1.54 ± 0.02% for isoflurane.11 End-tidal concentrations of halothane and isoflurane were measured using a Siemens Gas Analyzer 120 sampling from the expiratory limb of the circuit.

In both parts A and B, anesthesia was maintained for 30 min after surgical preparation was completed to allow the end-tidal concentrations of either N2O, halothane, or isoflurane to stabilize. Arterial blood was sampled for analysis of Pco2, Po2, and pH. Values of Po2 in the N2O plus halothane group were not reported due to a malfunction in the Po2 electrode during that part of the study.

Bupivacaine was then continuously infused at 1 mg·kg−1·min−1 using a mechanical pump. The threshold doses of bupivacaine were recorded for each of four events: 1) first ventricular dysrhythmia, 2) first seizure activity in the EEG, 3) isoelectric EEG, and 4) asystole. Asystole was defined as absence of a QRS complex on the ECG and absence of a pressure pulse on the arterial blood pressure trace. All animals developed asystole as the terminal event. Every 5 min during the infusion, cardiac output was determined noninvasively using the indirect Fick equation and a non-rebreathing technique.12 Cardiac index was calculated on the basis of body surface area (BSA), where BSA in m2 = 0.07 weight (kg)0.633. Every 5 min, in parts A and B, blood was sampled, and plasma was separated and frozen at −80°C for measurement of plasma concentrations of bupivacaine by high-performance liquid chromatography (HPLC) (linear over a range of 0.4 to 100 μg·ml−1; 6% coefficient of variation, interassay).13 Plasma concentrations of bupivacaine at which the four events occurred were interpolated from plasma concentrations before and after the events.

One-way analysis of variance (ANOVA) and the Student-Newman-Keuls test were used to compare the differences among the groups for plasma concentrations and threshold doses of bupivacaine, the times for terminal depression of cardiac index, and baseline values for cardiac index, systolic blood pressure, heart rate, and arterial blood gas tensions.

Results

Baseline blood gas values did not differ among the groups of parts A and B. Baseline Po2 values ranged from 94 to 173 mmHg, Pco2 values ranged from 31.2 to 32.5 mmHg, and pH values ranged from 7.41 to 7.47.

Part A

Plasma concentrations of bupivacaine were significantly higher in 2-day-old pigs than they were in 2-week-old or 2-month-old pigs at all times during infusion (fig. 1). The threshold doses of bupivacaine for dysrhythmias and seizures were higher in the 2-day-old pigs than in the 2-week-old and 2-month-old pigs (fig. 2). Ventricular dys-
rhythms occurred before seizures in each pig. The doses that produced isoelectric EEG and asystole did not differ significantly. The plasma concentrations of bupivacaine for all events were higher in the 2-day-old pigs than in the 2-week-old and 2-month-old pigs (fig. 3). Baseline cardiac index was higher in the 2-month-old pigs (mean CI ± SD = 5.25 ± 1.23 l·min⁻¹·m⁻²) than the 2-day-old pigs (mean CI ± SD = 2.15 ± 0.67 l·min⁻¹·m⁻²) and the 2-week-old pigs (mean CI ± SD = 3.08 ± 0.62 l·min⁻¹·m⁻²). Cardiac index was maintained in each pig until immediately before asystole despite seizures and dysrhythmias (fig. 4). Baseline systolic blood pressures (mean ± SD = 92.7 ± 12.7 mmHg for the 2-day-old pigs, 101.8 ± 25.4 mmHg for the 2-week-old pigs, and 124.8 ± 30.0 mmHg for the 2-month-old pigs) did not differ statistically and were maintained in a parallel fashion similar to cardiac index until immediately before asystole. Baseline heart rate in the 2-day-old pigs (mean HR ± SD = 156.7 ± 29.7 beats·min⁻¹) was slower than it was in the 2-week-old pigs (mean HR ± SD = 220.7 ± 23.9 beats·min⁻¹) and the 2-month-old pigs (mean HR ± SD = 208.0 ± 47.3 beats·min⁻¹).

### PART B

All pigs receiving N₂O alone or N₂O plus halothane had ventricular dysrhythmias, and the threshold doses for dysrhythmias did not differ between these two groups (fig. 5). Only one of the 2-day-old pigs receiving N₂O plus...
isoflurane had a dysrhythmia and only two had seizures. All of the pigs in the N₂O alone group had seizures, but none of the pigs receiving N₂O plus halothane had seizures. Doses required to produce isoelectric EEG and asystole were less in the halothane and isoflurane groups than in the N₂O alone group (fig. 5). The plasma concentrations of bupivacaine were higher at 5 min in the pigs receiving isoflurane (fig. 6) and at asystole in the pigs receiving halothane (fig. 7). Baseline cardiac indices (mean CI ± SD = 2.13 ± 0.67 l·min⁻¹·m² in the N₂O alone group, 2.02 ± 0.88 l·min⁻¹·m² in the N₂O plus halothane group, and 2.88 ± 0.19 l·min⁻¹·m² in the N₂O plus isoflurane group) did not differ statistically. During bupivacaine infusion, CI was preserved longer in the N₂O alone group compared with the N₂O plus isoflurane group and the N₂O plus halothane group (fig. 8). Baseline systolic blood pressure in the pigs receiving N₂O plus halothane (mean SBP ± SD = 57.0 ± 6.0 mmHg) was less than in the pigs receiving N₂O alone (mean SBP ± SD = 92.7 ± 12.7 mmHg) but was not statistically less than in the pigs receiving N₂O plus isoflurane (mean SBP ± SD = 83.5 ± 28.5 mmHg). Systolic blood pressure was maintained in a parallel fashion similar to CI until immediately before asystole. Baseline heart rates (mean HR ± SD = 156.7 ± 29.7 beats·min⁻¹ in the N₂O alone group, 148.0 ± 17.3 beats/min in the N₂O plus halothane group, and 139.5 ± 16.1 beats·min⁻¹ in the N₂O plus isoflurane group) did not differ statistically.

**Discussion**

Despite attaining higher plasma concentrations, the 2-day-old pigs in this study displayed greater resistance to the dysrhythmogenic and epileptogenic effects of bupivacaine than both the 2-week-old and 2-month-old pigs. Although the etiology is unclear, these observations agree with studies of lidocaine toxicity in adult, newborn, and fetal sheep. As in our study, younger animals required

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**Fig. 5.** Doses required to produce ventricular dysrhythmias (DYS), seizures (SZ), isoelectric electroencephalograph (ISO EEG), and asystole (ASYS) in 2-day-old pigs given N₂O/O₂, N₂O/O₂ with halothane, and N₂O/O₂ with isoflurane during infusion of bupivacaine 1 mg·kg⁻¹·min⁻¹. No seizures occurred in the halothane group. In the isoflurane group, only one of six pigs had a dysrhythmia and only two had seizures. Data are mean ± SD. *P < 0.05 (N₂O alone group compared with N₂O plus halothane and N₂O plus isoflurane).**

**Fig. 6.** Plasma concentrations achieved during infusion of bupivacaine 1 mg·kg⁻¹·min⁻¹ versus time in 2-day-old pigs given N₂O/O₂, N₂O/O₂ with halothane, and N₂O/O₂ with isoflurane. The number (n) of pigs diminished over time as the pigs died: for pigs given N₂O/O₂, n = 8 at 25 min, n = 4 at 55 min, n = 2 at 40 min, and n = 1 at 50 min; for pigs given N₂O/O₂ with halothane, n = 6 at 10 min, n = 5 at 15 min, and n = 4 at 20 min; for pigs given N₂O/O₂ with isoflurane, n = 6 at 20 min, n = 5 at 25 min, and n = 2 at 30 min. Data are mean ± SD. *P < 0.05 (N₂O plus isoflurane compared with N₂O alone and N₂O plus halothane).**

**Fig. 7.** Plasma concentration required to produce ventricular dysrhythmias (DYS), seizures (SZ), isoelectric electroencephalograph (ISO EEG), and asystole (ASYS) in 2-day-old pigs given N₂O/O₂, N₂O/O₂ with 0.5 MAC halothane, and N₂O/O₂ with 0.5 MAC isoflurane during infusion of bupivacaine 1 mg·kg⁻¹·min⁻¹. No seizures occurred in the halothane group. In the isoflurane group, only one of six pigs had dysrhythmias and only two had seizures. Data are mean ± SD. *P < 0.05 (N₂O plus halothane compared with N₂O alone and N₂O plus isoflurane).**
higher doses of local anesthetic agent than older animals to produce seizures. The lethal dose of bupivacaine in our study (i.e., the dose required to produce asystole), however, did not differ among the three age groups.

May the increased resistance to bupivacaine toxicity in younger pigs be explained on the basis of protein binding? If a significant amount of bupivacaine is bound to plasma proteins (α1-acid glycoprotein and albumin), less free fraction is available to exert toxic effects. Human neonates and infants have lower concentrations of α1-acid glycoprotein than older children. However, pigs that are 48 hours, 2 weeks, and 7 weeks old have similar serum α-globulin concentrations (1.2, 1.12, and 1.2 g·dl⁻¹, respectively). Because the lower concentrations of α1-acid glycoprotein in human infants would result in higher free drug concentrations, they may not be as resistant to bupivacaine toxicity as the young pigs.

At high serum concentrations of bupivacaine such as attained in this study, binding to serum albumin may assume greater importance. Although serum albumin concentrations in the human neonate may exceed those in the mother, 2-day-old pigs have approximately one-third the albumin concentration of older pigs. Therefore, the age differences in toxic doses of bupivacaine in pigs may be independent of both α1-acid glycoprotein and albumin binding.

There are data to substantiate that a more important influence than serum protein binding on the toxicity of bupivacaine in young animals may be the immaturity of the cardiovascular system of these animals. Compared with adult dogs, younger dogs require relatively higher doses of propranolol to slow the heart rate. These data may be explained by the relative immaturity of the sympathetic innervation of the heart described in biochemical and histochemical studies of fetal, neonatal, and adult rabbit myocardia.

Likewise, immaturity of the central nervous system may play a role in increased resistance to the toxic effects of local anesthetics in young animals. Compared with older rats, younger rats require a relatively higher dose of theophylline to produce seizures despite higher cerebrospinal fluid-to-brain concentrations at onset of seizures. The resistance to theophylline toxicity is not due to difference in serum protein binding of the drug but rather is a function of age, since the serum-free fraction of theophylline does not differ among groups. Similarly, in infants and children given iv morphine after surgery, respiratory depression does not differ among age groups despite blood levels (measured when pain returns) that are seven times higher in the youngest group.

The higher “plateau” of bupivacaine plasma concentration in the 2-day-old pigs as compared with the older pigs may reflect a substantially smaller steady state volume of distribution in the younger animals. On the other hand, the slower time to plateau in the 2-day-old animals suggests a slower movement of bupivacaine from the central compartment to other compartments as compared with the older animals. The progression of toxic signs in the presence of nearly constant venous plasma concentrations of bupivacaine suggests the presence of increasing accumulations of bupivacaine at target sites (especially the brain and myocardium).

Our methodology included the continuation of bupivacaine infusion until ECG evidence of asystole (during

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**Fig. 8.** Change in cardiac index as a percentage of control (baseline) versus time in 2-day-old pigs given N₂O/O₂ alone, N₂O/O₂ with halothane, and N₂O/O₂ with isoflurane during infusion of bupivacaine 1 mg·kg⁻¹·min⁻¹. Each line represents the data from one pig. The time for terminal cardiac depression was greater in the N₂O group *P ≤ 0.05.*
which time there was no cardiac output). This methodology would encourage pooling of bupivacaine in the vena cava prior to measurement of the bupivacaine concentration at asystole. Evidence of the pooling phenomenon is seen as terminal elevation of bupivacaine concentrations (fig. 6) and as a higher bupivacaine concentration in pigs receiving halothane who had a greater reduction in cardiac output (fig. 7).

When cerebral depression and cardiac arrest are the potential end-points of toxicity, it is clinically useful to be able to observe the early warning signs of seizures and dysrhythmias. The awake adult patient or the older child may verbalize the early signs of central nervous system toxicity (dizziness, perioral tingling, and tinnitus). In the anesthetized or pre-verbal child, however, dysrhythmias may be the only warning sign observed. In our study, pigs lightly anesthetized with N₂O alone always had dysrhythmias before seizures. In the pigs anesthetized with N₂O plus halothane, no seizures occurred, and in the pigs anesthetized with N₂O plus isoflurane, only one pig had a dysrhythmia and only two had seizures. These data suggest that cardiovascular toxicity from bupivacaine may occur without evidence of central nervous toxicity in infants and children anesthetized with N₂O and 0.5-MAC halothane. Furthermore, N₂O and 0.5-MAC isoflurane, but not N₂O and halothane, may prevent or attenuate the dysrhythmogenic effect of bupivacaine in infants and children.

Our data would further suggest that the margin of safety between the onset of dysrhythmias (when they occur) and life-threatening cardiovascular depression may be narrow in infants given bupivacaine during N₂O plus either halothane or isoflurane anesthesia. The onset of extrasystolic ventricular contractions may warn of impending cardiac depression in these children. The fact that blood pressure and cardiac output were maintained in our study until just before asystole may mean that systolic blood pressure will be maintained in these children despite impending cardiac arrest.

What does this study in pigs tell us about current dosage regimens in infants and children? Certainly, the toxic plasma concentrations of bupivacaine observed in our pigs are well above the plasma concentrations observed after either caudal administration of 2–3.75 mg·kg⁻¹ bupivacaine (0.5–1.2 μg·mL⁻¹) or interpleural administration of bupivacaine at 1.25 mg·kg⁻¹·h⁻¹ (2–7 μg·mL⁻¹) in infants and children and the plasma concentrations reported to be toxic in adults (3–5 μg·mL⁻¹). The relatively high plasma bupivacaine concentrations achieved before toxicity in our study and the relatively high concentrations achieved without toxicity after slow infusion into the interpleural space agree with data suggesting that the rate of infusion is a critical factor in determining local anesthetic toxicity.

It should be noted that our pigs had ventilation controlled and were normocarbic. Bupivacaine toxicity may occur earlier in spontaneously ventilating children anesthetized with a volatile agent because the hypercarbia that may develop would enhance dysrhythmogenicity. Furthermore, only 0.5-MAC halothane (0.48%) and isoflurane (0.77%) were used in our study. Higher concentrations of volatile agents may further enhance myocardial toxicity.

Our study most closely mimics the clinical situation where general anesthesia is administered before the injection of local anesthetic. In this situation, early warning signs of intravascular injection may be very important. Since all animals that received halothane had dysrhythmias prior to cardiovascular collapse, perhaps halothane would be a better choice than isoflurane (i.e., one could stop injecting at the onset of dysrhythmias). Similarly, all animals that received N₂O alone had seizures prior to collapse; however, we are not recommending avoidance of a potent anesthetic while performing local anesthetic injection. It may be more hazardous to attempt to administer a regional anesthetic in a struggling unanesthetized child than to risk the hazards of intravenous local anesthetic injection during volatile agent anesthesia.

In summary, we conclude that 1) toxic plasma concentrations of bupivacaine are age-dependent in young pigs, 2) 2-day-old pigs are more resistant than are 2-week-old and 2-month-old pigs to the toxic effects of bupivacaine, 3) N₂O plus halothane and N₂O plus isoflurane increase the lethality of bupivacaine while preventing or attenuating early warning neurologic signs (seizures), and 4) N₂O plus isoflurane, but not N₂O plus halothane, prevents or attenuates the dysrhythmogenic effect of bupivacaine.

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