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Effects of Imposed Acid–Base Derangement on the Cardiovascular Effects and Pharmacokinetics of Bupivacaine and Thiopental

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Background: **By changing physicochemical properties such as effective lipophilicity, changes in blood pH could alter the distribution, elimination, and effects of weakly ionizing drugs. The authors examined the outcome of imposed acid–base derangement on cardiovascular effects and myocardial and whole body pharmacokinetics of bupivacaine, a weak base, and thiopental, a weak acid.**

Methods: **Intravenous infusions of *rac*-bupivacaine HCl (37.5 mg) or *rac*-thiopental sodium (250 mg, subanesthetic dose) were administered over 3 min to previously instrumented conscious ewes with normal blood pH, acidemia imposed by lactic acid infusion, or alkalemia imposed by bicarbonate infusion. Hemodynamic and electrocardiographic effects were recorded; arterial and coronary sinus drug blood concentrations were analyzed by chiral high-performance liquid chromatography.**

Results: **Bupivacaine decreased myocardial contractility, coronary perfusion, heart rate, and cardiac output; however, cardiac output and stroke volume were not as affected by bupivacaine with acidemia. Thiopental decreased myocardial contractility and stroke volume and increased heart rate; acidemia enhanced the tachycardia and produced a greater decrease in stroke volume than with alkalemia. Taken as a whole, the cardiovascular changes were not systematically modified by acid–base derangement. Overall, the tissue distribution of bupivacaine was favored by alkalemia, but thiopental pharmacokinetics were essentially unaffected by acid–base derangement. Acid–base derangement did not influence the kinetics of either drug enantioselectively.**

Conclusions: **At the doses used, the hemodynamic and electrocardiographic effects of bupivacaine and thiopental were not systematically modified by acid–base derangement, nor were there changes in regional or whole body pharmacokinetics of either drug that were clearly related to acid–base status.**

EXCEPT for drugs that act through their metabolites, the time course of action of drugs on the heart generally relates closely to the time course of their concentrations in the heart.[1] Studies to ascertain the importance of controlling physiologic and biochemical factors such as perfusate pH, perfusion rate, and protein composition have mostly been performed in isolated perfused organ systems that allow the variables to be controlled separately. For example, studies using quinidine have found its cardiac tissue permeability and consequent cardiac effects to be principally dependent on unbound rather than total drug concentrations, but with significant tissue permeability to the ionized drug form.[2–4] More recent study designs have now allowed the regional myocardial pharmacokinetics and effects of cardioactive drugs to be described concomitantly in large animals.[5,6]

In this study, we examined the effects of imposed acidemia and alkalemia on pharmacokinetics and effects of two cardioactive drugs, bupivacaine (a weak base, pKa 8.1) and thiopental (a weak acid, pKa 7.6) in conscious sheep. Both bupivacaine and thiopental are susceptible to changes in extent of ionization through changes in local pH; decreasing the local pH increases the fractional ionization of bupivacaine and decreases the fractional ionization of thiopental, and *vice versa*. For both drugs, the relative concentration ratio of ionized to nonionized forms would change approximately twofold over the pH range used in the study. The significance of this is that nonionized forms are much more lipophilic than ionized forms and thus more readily diffuse into cells. Because the nonionized form is always in dynamic equilibrium across the membrane, if intracellular pH favors ionization more than does extracellular pH, net drug partitioning into the cytoplasm by "ion trapping" will be favored, resulting in a drug concentration gradient that is sensitive to a change in extracellular pH. The relative degrees of plasma protein and tissue binding also influence drug concentration gradients. Because of hydrophobic bonding, nonionized forms are generally more highly bound than ionized forms, but the effect of changes in local pH gradients on the equilibrium between plasma protein and tissue-bound drug, and therefore local drug concentration gradient, is generally not predictable from current knowledge. Therefore, it is possible that acid–base derangement, by affecting the extent of ionization of ionizable drugs, could influence their interactions with receptors and their pharmacologic effects, as well as their regional and whole body pharmacokinetics. Previous studies suggest that the myocardial effects of both bupivacaine and thiopental would be exacerbated by acidosis, although for bupivacaine, hypoxia also was required.[7,8]

Moreover, both bupivacaine and thiopental are used as racemates, *i.e.*, equal part mixtures of *R*- and *S*-enanti-

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omers. Previous studies have demonstrated enantioselectivity in pharmacokinetics and pharmacodynamics of these drugs, with *R*-bupivacaine and *S*-thiopental being considered the more toxic enantiomers.[9–13] Thus, the potential exists for acid–base derangement to cause differential pharmacologic effects through enantioselective pharmacokinetic changes.

Therefore, our objectives in this study were threefold: (1) to determine whether imposition of acid–base derangement altered the hemodynamic/inotropic/electrocardiologic effects of bupivacaine or thiopental, (2) to determine whether any such pharmacologic effects could be explained by changes in regional or whole body pharmacokinetics of these drugs or both, and (3) to determine whether acid–base derangement produced any enantioselective effects in the pharmacokinetics of these drugs.

Materials and Methods

Experimental Details

The study protocol was jointly approved by the Animal Care and Ethics Committee of the Royal North Shore Hospital, St. Leonards, Australia, and the University of Technology, Sydney, Australia. The subjects consisted of 13 nonpregnant Merino-cross ewes (age, 1–2 yr; weight [mean ± SD], 46 ± 5 kg); 11 subjects contributed data on bupivacaine, 9 contributed data on thiopental, and 7 contributed data on both drugs. Six additional subjects were used in pilot experiments. All subjects were vaccinated against clostridial diseases and caseous lymphadenitis and were drenched with the anthelmintic ivermectin before delivery to the animal house. They were allowed to acclimatize to their new environment for at least 1 week before surgical preparation for studies.

Preparation for Studies. Surgical preparation consisted of two stages, a left thoracotomy and cannulation of blood vessels performed during general anesthesia, at least 7 days apart. The thoracotomy involved placement of (1) active redirection transit time probes (Triton Technology, San Diego, CA) around the pulmonary trunk (21 mm) and left main coronary artery (6 mm) for respective measurement of cardiac output and left coronary artery blood flow; (2) a pressure transducer catheter (3-French, 100 cm, model SPR-524; Millar Instruments, Houston, TX) transmurally into the left ventricle to provide left ventricular pressure, from which the maximum value of its positive first derivative (LV dP/dt_{max}) was determined as an index of myocardial contractility; (3) a polyurethane catheter (45-cm, 16- or 18-gauge Cavafix Certo 358 or 255; B Braun Melsungen AG, Melsungen, Germany) in the coronary sinus for blood sampling; and (4) electrodes made from fishing wire (20-pound nylon-coated stainless steel) around the dorsal and ventral extremities of the fifth rib to acquire electrocardiographic signals. The left hemiazygos vein, which drains into the coronary sinus in the sheep, was ligated to prevent contamination of coronary sinus blood samples with systemic venous blood.

At surgery for cannulation, two polyurethane catheters (16- or 18-gauge, 45-cm, Cavafix Certo 358 or 255) for measurement of arterial blood pressure and sampling of arterial blood were placed in the left carotid artery to the brachiocephalic trunk. A double-lumen central venous catheter (70-cm Cavafix Duo, B Braun Melsungen AG) was placed *via* the jugular vein for administration of drugs. After recovery, cannulated sheep were kept in metabolic crates so that patency of catheters could be maintained by continuous flushing with heparinized (5 U/ml) saline (0.9%) at approximately 3 ml/h per catheter by a high-pressure infusion system.

Flucloxacillin (1 g intravenous) was given before incision and hourly during surgery as prophylaxis against infection. Postoperative analgesia was provided with 0.5% bupivacaine by blockade, during chest closure, of the intercostal nerves cranial and caudal to the thoracotomy incision, along with carprofen (75 mg intravenous) and buprenorphine (0.3 mg intravenous) after anesthetic induction, during anesthetic recovery, and also two or three times daily for approximately 4 days thereafter.

Dosage Regimens and Treatments. A period of 4–7 days was left between the second surgery and the first study in accord with the recovery of the animals. Studies were performed with the animals conscious and supported by a sling in a metabolic crate to prevent alteration of their position relative to the monitoring system and to support their weight if they wanted to relax.

Five treatments were studied: test drug infusion with normal arterial pH, with acidemia from intravenous infusion of lactic acid, and with alkalemia from intravenous infusion of sodium bicarbonate; in addition, acidemia and alkalemia control studies were performed in which saline (0.9%) was substituted for the test drug. The test drugs consisted of *rac*-bupivacaine HCl (37.5 mg) or *rac*-thiopental sodium (250 mg) infused intravenously over 3 min at 10 ml/min. Based on our previous experiments, the dosage regimen of bupivacaine was expected to be subconvulsive and that of thiopental was expected to be subanesthetic; both doses, however, were expected to cause significant myocardial depression. Bupivacaine studies preceded thiopental studies.

The regimens used to produce acidemia and alkalemia were determined empirically in pilot studies. Acidemia was produced by infusing 15% *rac*-lactic acid at 10 ml/min for 5 min, 5 ml/min for 15 min, then 2.5 ml/min for 25 min; infusion of the test drug or saline began 5 min after the start of the lactic acid infusion. Alkalemia was produced by infusing 8.4% sodium bicarbonate at 13 ml/min for 25 min, then 7.5 ml/min for 25 min; infusion of test drug or saline began 10 min after the start of bicarbonate infusion. For studies with normal blood

pH, saline (0.9%) was infused with the same regimen as for the bicarbonate infusion.

Blood Sampling Regimen. Samples of arterial and coronary sinus blood were collected into heparinized syringes just before the start of the infusions to change blood pH and then before and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 7.5, 10, 15, 20, 30, 40, 45, and 60 min after the start of infusion of test drug or saline. Further arterial samples were taken at 90, 120, 150, and 180 min to better characterize the whole body pharmacokinetics. The samples were stored at −20°C until analysis of drug concentration by chiral high-performance liquid chromatography.[14,15] Arterial blood gases and acid–base balance were analyzed in samples drawn before and 1, 3, 5, 10, 20, 30, and 60 min after the start of infusion of drug or saline.

Data Acquisition and Processing

Hemodynamic and Electrocardiographic Data. Analog data were acquired and stored digitally by a personal computer interfaced with a physiologic monitoring system (Triton System 6; Triton Technology, San Diego, CA) and data acquisition/analysis system (MP100 with Acqknowledge v3 software; Biopac Inc., Santa Barbara, CA) at a sample rate of 256 Hz.[16] Heart rate was obtained from the electrocardiographic trace, and stroke volume was obtained from the quotient of cardiac output and heart rate. The monitoring systems were calibrated, and baseline data were collected for 5 min. Data were acquired for 3 h after the start of infusion of drug or saline. Hemodynamic data were collected as the mean values of 30-s epochs, except for LV dP/dt_{max}, which was collected as the maximum value in the epoch.

Mean arterial blood pressure, LV dP/dt_{max}, heart rate, cardiac output, and left coronary artery blood flow showed a baseline drift with alkalemia, so an automatic baseline correction algorithm (see Appendix) was designed and incorporated into the spreadsheet used for data maintenance (Excel 97; Microsoft, Redmond, WA). This correction was applied to all data sets for consistency; however, its effects were negligible for the normal pH and acidemia data sets.

The electrocardiogram was analyzed for arrhythmias and measurements were made of time between beginning of P wave to beginning of Q wave (or R wave if no Q wave was present) (P to R wave interval), time from onset of Q wave to end of T wave (Q to T wave interval), time between consecutive R waves (R to R wave interval), and width of the electrocardiographic wave complex (QRS width); also, the Q to T wave interval was corrected for heart rate by dividing it by the square root of the R to R wave interval (corrected Q to T wave interval [QT_c]). For these measurements, means were taken of three consecutive complexes that were not part of an arrhythmia in the 10 s immediately preceding the following times: during the control period at 1, 2, 3, 4, and 5 min; at 10 and 15 min while acid–base balance was being changed; and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7.5, 10, 15, 20, 30, 45, and 55 min after the start of drug infusion. Cardiac arrhythmias during the first 10 min of saline or drug infusion were grouped into supraventricular arrhythmias (supraventricular premature beats, supraventricular tachycardia, supraventricular bigeminy) and ventricular arrhythmias (ventricular premature beats, ventricular tachycardia, ventricular bigeminy, ventricular trigeminy).

Hemodynamic and electrocardiographic data were analyzed for the magnitude of greatest effect (E_{max} or E_{min}) and its time of occurrence. In addition, the sum of the effect differences to 10 min for cardiovascular and hemodynamic data and to 5 min for electrocardiographic data were determined to capture any differences between treatments in magnitude and immediate time course attributable to drug effect.[16] These data provide useful univariate statistics for time series data that are analogous to areas under the curves.

Statistical Analyses

Statistix for Windows (version 7.1; Analytical Solutions, Tallahassee, FL) was used on a personal computer. Cardiovascular and pharmacokinetic data were analyzed by two-factor analysis of variance with treatment the main effect and subject a repeated factor. The null hypothesis was that there was no difference in effects between treatments. A criterion of $P < 0.05$ was taken as weak evidence for rejection of the null hypothesis, and $P < 0.01$ was taken as strong evidence. If there was a significant effect of treatment, differences in mean values between treatment groups were determined by the method of least significant differences.

Results in graphic form are reported as mean ± SEM to show the predicted variability of the mean values. Tabulated results are reported as mean with 95% confidence interval. The absolute values for the relevant pretreatment predrug (baseline) cardiovascular parameters, along with maximum after-drug changes (either E_{max} or E_{min}, as appropriate, expressed as percent of baseline values) are also given. Note that after-drug 95% confidence intervals that do not include 100% are significant changes ($P < 0.05$).[17] All tests were two tailed. The frequency of subjects having cardiac arrhythmias with each treatment was compared by chi-square analysis.

The effects of acid–base derangement on whole body pharmacokinetics were explored sequentially. First, each drug was treated as a racemate (*i.e.*, the same as the dosage form) by summing the respective measured blood concentrations of the enantiomers. Each set of arterial blood drug concentration–time data was fitted with a triexponential decay equation by an iterative nonlinear weighted least squares procedure decided on the basis of minimal objective function and of symmetry of residuals.[18] From the equation of best fit, relevant

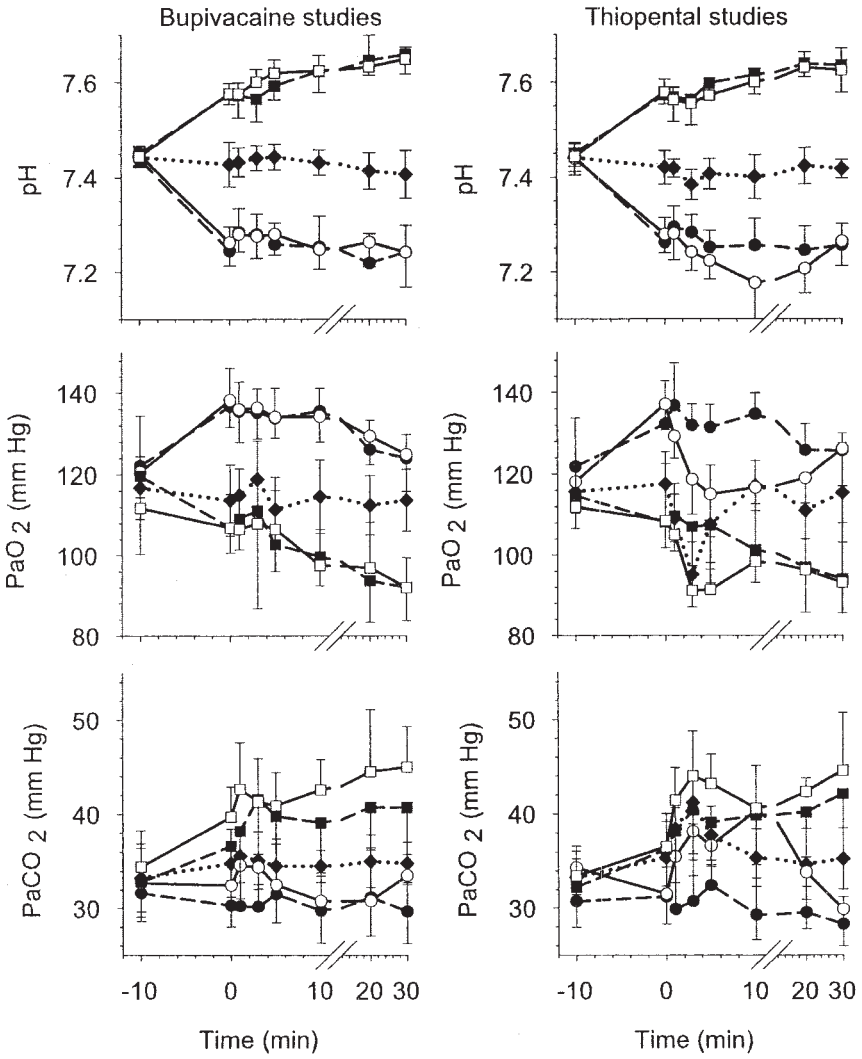

Fig. 1. Time course of changes in arterial blood pH and gases in studies with intravenous infusion of bupivacaine (HCl salt, 37.5 mg/3 min) or thiopental (sodium salt, 250 mg/3 min) with normal arterial pH (*closed diamonds*), imposed acidemia (*closed circles*) or alkalemia (*closed squares*); acidemia (*open circles*) or alkalemia (*open squares*) without drug are also shown. Time 0 denotes commencement of drug or saline infusion. Note time scale expansion for clarity.

pharmacokinetic properties of a three-compartment open model (initial dilution volume [V_c], total apparent volume of distribution [V_{ss}], mean total body clearance [CL_t], and slow half-life [T1/2γ]) were determined. The effect of acid–base derangement on the pharmacokinetic properties was determined by two-factor (subject × treatment) analysis of variance. Second, the pharmacokinetics of the separate enantiomers were determined as above.[18] Using the Student one-sample *t* test, enantioselectivity was assessed by comparing, to a value of unity, the ratio of the value for the *R*-enantiomer to the corresponding value for the *S*-enantiomer (*R*:*S* ratio) of the respective relevant pharmacokinetic properties. Because the enantiomer concentrations were measured in each blood sample, this ratio reflects the within-subject pharmacokinetic variability. The effect of acid–base derangement on the *R*:*S* ratio of each relevant pharmacokinetic parameter was determined by two-factor (subject × treatment) analysis of variance. Third, regional (myocardial) pharmacokinetics were determined from the concentrations of drug (as racemate) in paired arterial and coronary sinus blood samples and coronary arterial blood flow. Because some sampling catheters and probes failed during the study period, paired data were available for only four subjects with each drug and treatment; hence, calculations of myocardial kinetics by mass balance were based on mean drug concentration–time–blood flow data.[5,6,10]

Comparisons between bupivacaine and thiopental were not contemplated because the principal objectives were to determine whether the pharmacodynamics and pharmacokinetics of each drug were altered by acid–base derangement.

Results

Arterial Blood Gases and Acid–Base Balance

Lactic acid infusions decreased arterial blood pH to approximately 7.25, and sodium bicarbonate infusions increased it to approximately 7.65, with and without concurrent infusions of bupivacaine and thiopental (fig. 1). Concurrently, arterial oxygen tension (Pa_{O_2}) tended to increase with lactic acid and decrease with bicarbonate infusions; conversely, arterial carbon dioxide tension

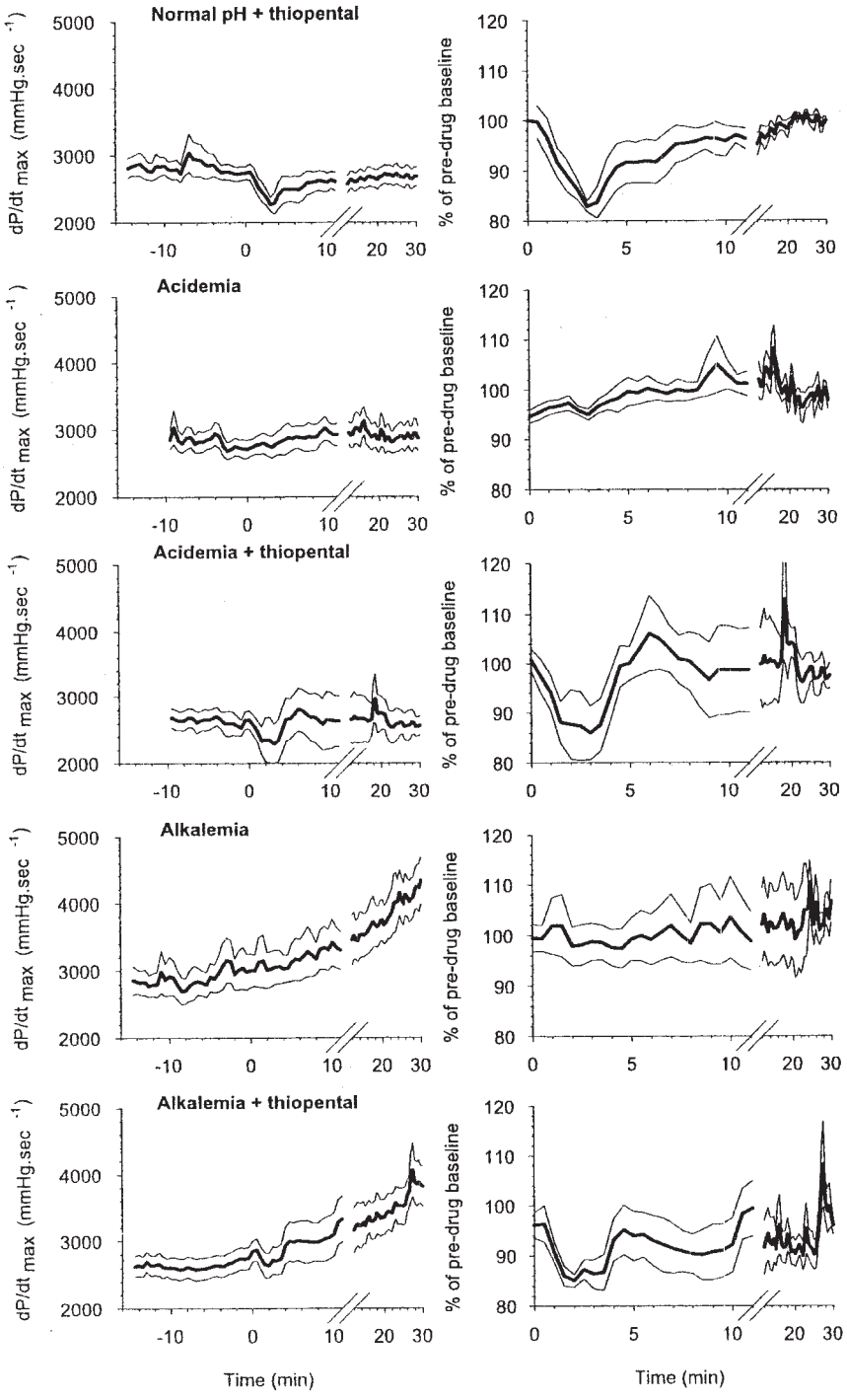

Fig. 2. Time course of changes to left ventricular dP/dt_{max} with intravenous infusion of thiopental (sodium salt, 250 mg/3 min) under conditions of normal arterial pH, imposed acidemia, or alkalemia. Effects of imposed acidemia or alkalemia without drug are also shown. Data are shown as mean (*center thick lines*) ± SEM (*outside thin lines*) of absolute raw data (*left panels*) and percent of baseline after automatic baseline correction (*right panels*). Time 0 denotes commencement of drug or saline (control) infusion. Note time scale expansion for clarity.

($Paco_2$) tended to increase with bicarbonate and decrease with lactic acid infusion. However, the animals were neither hypoxemic nor hypercapnic during the studies.

Hemodynamic and Electrocardiographic Effects

Mean within-subject predrug values did not differ between treatments with the one exception of QT_c interval with thiopental (see discussion of effects of thiopental later in this section). Figure 2 shows the representative time course of effects for raw data and for automatic baseline-corrected data (expressed as percent of baseline) for LV dP/dt_{max} with thiopental infusion. With normal arterial pH, both bupivacaine and thiopental always produced maximum drug-induced changes within 5 min with recovery by 10–15 min. The maximum changes (E_{max} or E_{min}) paralleled the changes in sums of effect differences values, which accounted for differences in the time courses of effects; therefore, for brevity, sums of effect differences data are not shown.

Table 1. Cardiovascular System Parameters and Effects of Acid–Base Treatment ± Bupivacaine

Variable/Units	Treatment D: Normal pH + Bupivacaine (significance)*	Treatment A: Acidemia	Treatment AD: Acidemia + Bupivacaine	Treatment B: Alkalemia	Treatment BD: Alkalemia + Bupivacaine	2-factor ANOVA (significance of acid-base treatment)†
LV dP/dt_{max}						
Predrug, $mmHg \cdot s^{-1} \cdot 10^{-3}$	3.10 (2.29–3.93)	2.74 (2.34–3.15)	2.90 (2.45–3.32)	3.05 (2.44–3.66)	2.92 (2.39–3.44)	NSD
Max change E_{min}, %	80 (64–97; $P = 0.031$)	98 (95–100)	81 (74–88)	95 (90–99)	83 (75–90)	$P = 0.0001$ B = A; BD = D = AD
MABP						
Predrug, mmHg	84 (80–89)	90 (83–97)	87 (81–93)	85 (77–93)	86 (81–91)	NSD
Max change E_{max}, %	118 (106–130; $P = 0.01$)	104 (102–106)	111 (105–117)	106 (102–110)	119 (111–126)	$P = 0.0029$ BD = B = AD; AD = B = A
HR						
Predrug, beats/min	90 (79–102)	85 (73–97)	82 (73–90)	86 (75–97)	85 (75–95)	NSD
Max change E_{min}, %	89 (86–92; $P < 0.0001$)	97 (94–101)	91 (87–95)	95 (92–98)	88 (84–93)	$P = 0.0003$ A = B; B = AD; AD = D; AD = D = BD
CO						
Predrug, l/min	5.0 (3.6–6.5)	5.0 (3.7–6.3)	5.7 (3.9–7.4)	4.8 (3.9–5.6)	5.4 (4.1–6.6)	NSD
Max change E_{min}, %	83 (76–91; $P = 0.001$)	98 (90–105)	91 (89–93)	95 (92–98)	81 (73–88)	$P = 0.0001$ A = B = AD > D = BD
SV						
Predrug, ml	56 (40–73)	59 (43–76)	66 (49–84)	55 (45–64)	62 (49–74)	NSD
Max change E_{min}, %	84 (76–94; $P = 0.006$)	95 (87–103)	95 (90–100)	98 (96–101)	86 (81–92)	$P < 0.0001$ A = B = AD > D = BD
CABF						
Predrug, ml/min	300 (120–480)	255 (125–385)	277 (177–379)	255 (98–412)	298 (107–489)	NSD
Max change E_{min}, %	86 (75–98; $P = 0.029$)	97 (89–105)	96 (88–103)	85 (68–105)	86 (69–103)	NSD

Mean values (and lower to upper 95% CIs) are given.

* Those 95% CI values for maximum change that do not include 100% are significant, and exact probabilities are given. † Strings separated by semicolons indicate groups within whose mean values cannot be shown to be different.

CABF = left coronary artery blood flow; CO = cardiac output; E_{max} = maximum effect; E_{min} = minimum effect; HR = heart rate; LV dP/dt_{max} = first derivative of left ventricular pressure; MABP = mean arterial blood pressure; Max = maximum; NSD = no significant treatment effect; SV = stroke volume.

The effects of bupivacaine are given in tables 1 and 2. Bupivacaine caused central nervous system (CNS) excitation in three subjects with normal arterial pH and with alkalemia but not with acidemia. When arterial pH was normal, bupivacaine decreased LV dP/dt_{max}, heart rate, cardiac output, stroke volume, and left coronary artery blood flow and increased mean arterial blood pressure. For all variables except left coronary artery blood flow, there were significant differences between treatment groups. Although some variables showed significant changes with all treatments (*e.g.*, decreased LV dP/dt_{max} and increased mean arterial blood pressure), the changes with bupivacaine infusion were greater. Acidemia attenuated the decrease in cardiac output and stroke volume induced by bupivacaine, but overall, the hemodynamic effects of bupivacaine were not materially modified by changes in arterial pH. Bupivacaine with normal arterial pH also produced small but significant electrocardiographic changes; however, there were no differences related to treatment except for Q_Tc, for which the differences were small.

The effects of thiopental are given in tables 3 and 4. When arterial pH was normal, thiopental decreased LV dP/dt_{max} and stroke volume and increased heart rate and cardiac output. Acidemia enhanced the increased heart rate and also produced a greater decrease in stroke volume than with alkalemia; however, changes in cardiac output and LV dP/dt_{max} were unaffected by acidemia or alkalemia. Thiopental with normal arterial pH also produced small but significant electrocardiographic changes; however, there were no significant differences related to treatment. There were small differences in the pretreatment values of Q_Tc ($P = 0.04$), but there was no material difference in outcome of statistical analyses that ignored this difference or treated predrug value of Q_Tc as a covariate.

Supraventricular and ventricular arrhythmias were found in some subjects with both drugs and all treat-

Table 2. Electrocardiologic Effects of Acid–Base Treatment ± Bupivacaine

Variable/Units	Treatment DT: Normal pH + Bupivacaine (significance)*	Treatment A: Acidemia	Treatment AD: Acidemia + Bupivacaine	Treatment B: Alkalemia	Treatment BD: Alkalemia + Bupivacaine	2-factor ANOVA (significance of acid-base treatment)†
PR interval						
Predrug, ms	101 (89–117)	106 (90–122)	110 (96–124)	107 (89–124)	105 (89–122)	NSD
Max change E_{max}, %	115 (109–121; $P = 0.0004$)	113 (100–127)	121 (108–135)	110 (99–121)	112 (101–124)	NSD
QRS width						
Predrug, ms	69 (62–75)	65 (63–68)	67 (63–71)	64 (62–67)	67 (63–71)	NSD
Max change E_{max}, %	109 (104–114; $P = 0.0034$)	108 (102–114)	114 (104–124)	106 (101–111)	107 (103–112)	NSD
Q_{Tc} interval						
Predrug, ms	12.2 (11.5–12.8)	12.1 (11.4–12.8)	12.3 (11.6–12.8)	12.0 (11.2–12.6)	11.0 (11.2–12.6)	NSD
Max change E_{max}, %	104 (102–107; $P = 0.0085$)	105 (101–109)	111 (106–116)	107 (104–110)	110 (105–115)	$P = 0.0061$ AD = BD = B; BD = B; B = A = D

Mean values (and lower to upper 95% CIs) are given.

* Those 95% CI values for maximum change that do not include 100% are significant, and exact probabilities are given. † Strings separated by semicolons indicate groups within whose mean values cannot be shown to be different.

NSD = no significant treatment effect.

Table 3. Cardiovascular System Parameters and Effects of Acid–Base Treatment ± Thiopental

Variable/Units	Treatment T: Normal pH + Thiopental (significance)*	Treatment A: Acidemia	Treatment AT: Acidemia + Thiopental	Treatment B: Alkalemia	Treatment BT: Alkalemia + Thiopental	2-factor ANOVA (significance of acid-base treatment)†
dP/dt_{max}						
Predrug, $mmHg \cdot s^{-1} \cdot 10^{-3}$	2.86 (2.45–3.26)	2.77 (2.43–3.10)	2.63 (2.30–2.94)	2.85 (2.22–3.45)	2.49 (2.13–2.84)	NSD
Max change E_{min}, %	81 (75–86; $P = 0.0001$)	96 (91–101)	81 (67–94)	96 (91–101)	86 (79–92)	$P = 0.0003$ B = A > BT = AT = T
MABP						
Predrug, mmHg	90 (78–102)	90 (83–98)	90 (81–98)	87 (82–93)	85 (76–94)	NSD
Max change E_{min}, %	98 (96–100; $P = 0.045$)	98 (96–100)	101 (93–109)	100 (96–104)	96 (92–99)	NSD
HR						
Predrug, beats/min	95 (81–108)	90 (77–103)	84 (77–92)	91 (79–102)	91 (79–104)	NSD
Max change E_{max}, %	122 (116–127; $P < 0.0001$)	107 (103–110)	132 (120–143)	102 (98–106)	119 (114–124)	$P < 0.0001$ AT > T = BT > A = B
CO						
Predrug, l/min	6.8 (3.9–9.7)	4.9 (3.5–6.3)	6.1 (4.2–8.1)	5.5 (3.7–7.3)	6.1 (4.2–7.9)	NSD
Max change E_{min}, %	105 (101–109; $P = 0.021$)	113 (107–120)	107 (98–116)	105 (101–110)	105 (101–109)	NSD
SV						
Predrug, ml	71 (47–91)	56 (39–74)	73 (50–96)	61 (42–80)	68 (48–86)	NSD
Max change E_{min}, %	80 (76–85; $P < 0.0001$)	101 (97–105)	82 (70–94)	98 (94–102)	89 (84–95)	$P < 0.0001$ A = B > BT > AT = T
CABF‡						
Predrug, ml/min	242 (68–416)‡	285 (136–434)	265 (111–420)	284 (43–525)	222 (75–368)	NSD

Mean values (and lower to upper 95% CIs) are given.

* Those 95% CI values for maximum change that do not include 100% are significant, and exact probabilities are given. † Strings separated by semicolons indicate groups within whose mean values cannot be shown to be different. ‡ Did not change.

CABF = left coronary artery blood flow; CO = cardiac output; E_{max} = maximum ventricular elastance; E_{min} = minimum ventricular elastance; HR = heart rate; LV dP/dt_{max} = first derivative of left ventricular pressure; MABP = mean arterial blood pressure; Max = maximum; NSD = no significant treatment effect; SV = stroke volume.

Table 4. Electrocardiologic Effects of Acid–Base Treatment ± Thiopental

Variable/Units	Treatment T: Normal pH + Thiopental (significance)*	Treatment A: Acidemia	Treatment AT: Acidemia + Thiopental	Treatment B: Alkalemia	Treatment BT: Alkalemia + Thiopental	2-factor ANOVA (significance of acid-base treatment)†
PR interval						
Predrug, ms	112 (94–129)	114 (96–132)	109 (91–128)	114 (97–132)	109 (90–128)	NSD
Max change E_{min}, %	93 (88–99; $P = 0.0001$)	93 (88–99)	91 (83–99)	94 (90–100)	89 (76–102)	NSD
QRS width						
Predrug, ms	63 (60–66)	65 (63–67)	63 (61–64)	64 (62–66)	65 (62–684)	NSD
Max change E_{min}, %	108 (104–111; $P = 0.045$)	105 (103–107)	107 (100–114)	104 (102–108)	107 (103–110)	NSD
Q_{Tc} interval						
Predrug, ms	12.9 (11.9–13.8)	12.1 (11.4–12.8)	12.4 (11.7–13.2)	12.0 (1.3–12.7)	12.3 (11.5–13.2)	$P = 0.04$ BT = AT = B; AT = B = A; B = A = T
Max change E_{max}, %	109 (104–113; $P < 0.0001$)	105 (100–111)	111 (104–118)	107 (104–110)	107 (104–111)	NSD

Mean values (and lower to upper 95% CIs) are given.

* Those 95% CI values for maximum change that do not include 100% are significant, and exact probabilities are given. † Strings separated by semicolons indicate groups within whose mean values cannot be shown to be different.

NSD = significant treatment effect.

ments; however, there was no difference in frequency of any arrhythmia according to treatment with either drug.

Whole Body Pharmacokinetics

Summed enantiomer drug blood concentrations are shown in figure 3. Pharmacokinetic analysis based on *rac*-bupivacaine found differences between treatments in V_{ss} and T1/2γ (table 5). Pharmacokinetic analysis as separate enantiomers, followed by determination of *R*:*S* ratios for the respective parameters, did not show pharmacokinetic enantioselectivity with any treatment. In contrast, pharmacokinetic analysis as *rac*-thiopental showed no differences between treatments (table 6), but there was significant pharmacokinetic enantioselectivity in CL_t and T1/2 with all treatments but no differences in enantioselectivity between treatments.

Myocardial Pharmacokinetics and Pharmacodynamics

No trends related to treatment were found when the effects on LV dP/dt_{max} were plotted against either arterial or coronary sinus blood concentration of either drug. Similarly, neither the arterial:coronary sinus blood concentration ratio nor the myocardial net flux differed according to treatment for either drug.

Discussion

This study found that imposed acid–base derangements did not systematically alter the effects of bupivacaine or thiopental on cardiovascular function in healthy sheep. Although the findings are largely negative, there is a positive message in that acidemia, which is considered by many to amplify the cardiovascular toxicity of bupivacaine, attenuated some of its adverse effects. However, for thiopental, some of the adverse effects were worsened by acidemia. In addition, bupivacaine caused CNS excitation only during alkalemia or when arterial pH was normal, and this is consistent with the observation that transport of lipophilic amines across the blood–brain barrier is inhibited by acidemia.[19]

Effects of directly acting cardioactive drugs, in general, depend on their concentrations in the heart, presumably at particular subsites, and these concentrations depend on both blood and tissue pH, which influence the rate of drug transfer between blood and tissue. Apart from dose and dosing rate, a number of factors can significantly affect the short-term concentration profiles of drugs in the heart. From a mass balance viewpoint, the concentration–time course of drug in this organ is driven by its blood flow and arterial blood-tissue concentration gradient of unbound drug. These factors are, in turn, driven by the physiologic status of microcirculation, oxygenation, and blood-tissue pH difference in the organ, as well as the drug's tissue affinity for and rate of elution from the organ, the latter again being determined by the organ blood flow and tissue-venous blood drug concentration gradient.[20] Although much has been learned from studying drugs with isolated organ systems *in vitro*,[2–4] there is still insufficient information relating regional blood flow, blood pH, and pharmacokinetics and pharmacodynamics of drugs in the heart *in vivo*. Such data can be obtained by using an appropriate large animal model. This chronically instrumented conscious sheep preparation provides a way of obtaining high-fidelity measures of relevant drug effects (*e.g.*, negative inot-

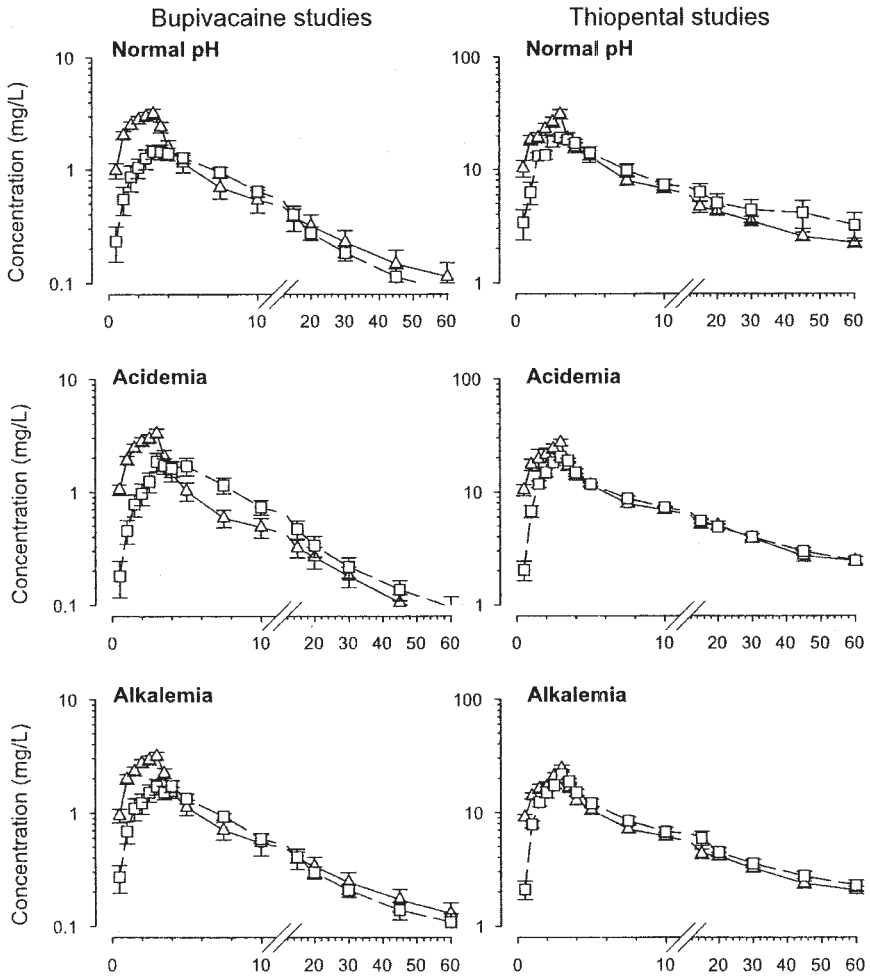

Fig. 3. Concentrations of bupivacaine (*left panels*) and thiopental (*right panels*) in arterial (*open triangles*) and coronary sinus (*open squares*) blood from intravenous infusion of bupivacaine (HCl salt, 37.5 mg/3 min) or thiopental (sodium salt, 250 mg/3 min) under conditions of normal arterial pH, imposed acidemia, or alkalemia. Note time scale expansion for clarity.

ropy) in concert with whole body and regional pharmacokinetics. Measurement of drug concentrations in samples of affluent and effluent blood of relevant organs (*e.g.*, for the heart, arterial and coronary sinus blood), combined with continuous measurement of organ blood flow, allows calculation of rate of drug uptake and elution therein using mass balance principles. Hence, effects of drugs in organs can be related to drug concentrations in those organs.[20]

It is difficult if not impossible to reproduce the clinical conditions of acid–base derangements in normal healthy subjects without foregoing some physiologic fidelity, introducing other perturbations, or both. Acid–base derangement was produced reliably, but the regimens were not free from potential problems. For example, the use of 8.4% sodium bicarbonate to increase pH would inevitably have increased blood tonicity also, resulting in increased blood volume by movement of water from cells, cardiovascular changes, and diuresis. Both cardiovascular changes and high urine output were observed.

Both bupivacaine and thiopental are myocardial depressants and, under certain circumstances, can inhibit, initiate, and/or support cardiac arrhythmias.[7,8,21–25] To avoid the opposing effects of CNS stimulation on the myocardial effects of bupivacaine, we chose what we thought, on the basis of our previous experiments, would be a subconvulsive dose.[9] However, as noted above, this dose was sufficient to cause some CNS excitation in three subjects when arterial pH was normal and during alkalemia; nevertheless, analysis with and without these animals removed from the data pool gave essentially the same results.

The pH changes in this study would have markedly changed the extent of ionization of these two drugs. At normal arterial pH, bupivacaine is predominantly ionized such that the concentration ratio of ionized:nonionized forms is 6.3:1; at pH 7.25, the ratio increases to 8.9:1, and at pH 7.65, it decreases to 3.6:1. Conversely, thiopental is predominantly nonionized at pH 7.40 with a concentration ratio of ionized:nonionized forms of 0.63:1; at pH 7.25, this decreases to 0.45:1, and at pH 7.65, it increases to 1.1:1. These changes have the potential to alter drug distribution, clearance, and effects because the nonionized form is much more lipophilic than the ionized form.[26] Although the enantiomers of these two drugs are treated as separate but interacting entities in the chiral environment of the body, the changes in extent of ionization predicted by the Hender-

Table 5. Summary of Bupivacaine Whole Body Pharmacokinetics in Sheep According to Treatment

Variable	Normal pH (B)	Acidemia (AB)	Alkalemia (BB)	2-factor ANOVA (significance of acid-base treatment)*
V_c, l	3.8 (3.1–4.5)	3.8 (3.1–4.5)	4.1 (3.5–4.7)	NSD
V_c R:S ratio	0.99 (0.98–1.01)	1.14 (0.93–1.34)	0.98 (0.93–1.02)	NSD
V_{ss}, l	50.3 (38.2–62.4)	52.0 (35.8–68.3)	70.6 (50.0–91.1)	$P = 0.0001$ BB > B = AB
V_{ss} R:S ratio	1.10 (0.97–1.24)	1.15 (0.84–1.47)	1.15 (0.87–1.43)	NSD
Cl_t, l/min	1.56 (1.14–1.98)	1.74 (1.15–2.32)	1.46 (1.10–1.79)	NSD
Cl_t R:S ratio	1.22 (0.99–1.45)	1.21 (0.97–1.45)	1.13 (0.99–1.27)	NSD
$T_{1/2\gamma}$, min	46 (37–54)	41 (27–56)	65 (36–94)	$P = 0.009$ BB = AB; B = AB
$T_{1/2\gamma}$ R:S ratio	1.02 (0.85–1.20)	1.04 (0.87–1.22)	1.15 (0.80–1.50)	NSD

A dose of 37.5 mg *rac*-bupivacaine HCl was infused intravenously over 3 min. Data are presented as mean (and 95% CIs). Pharmacokinetic values are given for *rac*-bupivacaine: Cl_t = mean total body clearance; $T_{1/2\gamma}$ = slow half-life; V_c = initial dilution volume; V_{ss} = total (steady-state) apparent distribution volume. Values for the R:S ratio of the pharmacokinetic values in the same experiment reflect within-animal values and are tested against a value of unity: 95% CI values of the ratio that do not contain unity indicate significant enantiomeric bias.

* Strings separated by semicolons indicate groups whose mean values cannot be shown to be different.

NSD = not significantly different between acid-base treatment groups; otherwise, significance of difference is shown.

son-Hasselbalch equation are the same for both enantiomers. The plasma protein binding of both drugs is affected by pH: Bupivacaine binding is appreciably decreased with decreased plasma pH[27,28] and thiopental binding is modestly increased with increased plasma pH.[29] However, their binding is also modestly enantioselective: both *R*-bupivacaine and *R*-thiopental undergo slightly greater plasma binding than their respective *S*-enantiomers in sheep.[13,30] Through physicochemical changes, altered pH could thus affect membrane permeability, tissue:plasma distribution, regional uptake and elution, whole body drug disposition, and metabolite activity, as well as receptor-related activity of these drugs.

Despite the potential of altered pH to modify pharmacodynamics, acid–base derangement did not systematically materially alter the overall effects of either drug, except for an amelioration by acidemia of decreased stroke volume and cardiac output induced by bupivacaine. Nor did acid–base derangement systematically alter pharmacokinetics, except for an increased total distribution volume (V_{ss}) with bupivacaine, which, with

Table 6. Summary of Thiopental Whole Body Pharmacokinetics in Sheep According to Treatment

Variable	Normal pH (T)	Acidemia (AT)	Alkalemia (BT)	2-factor ANOVA (significance of acid-base treatment)
V_c, l	2.7 (1.2–4.3)	2.1 (1.6–2.6)	2.5 (2.0–3.1)	NSD
V_c R:S ratio	0.99 (0.98–1.00; $P = 0.04$)	0.99 (0.98–1.00)	1.00 (0.99–1.01)	NSD
V_{ss}, l	43.3 (27.6–59.0)	38.2 (31.8–44.6)	46.6 (35.4–57.9)	NSD
V_{ss} R:S ratio	0.93 (0.88–0.98; $P = 0.009$)	0.87 (0.72–1.01)	0.90 (0.85–0.94; $P = 0.001$)	NSD
Cl_t, l/min	0.47 (0.41–0.53)	0.48 (0.38–0.59)	0.51 (0.44–0.57)	NSD
Cl_t R:S ratio	1.34 (1.29–1.37; $P < 0.0001$)	1.42 (1.31–1.54; $P = 0.0001$)	1.41 (1.31–1.51; $P = 0.0001$)	NSD
$T_{1/2\gamma}$, min	79 (60–98)	76 (47–104)	78 (66–90)	NSD
$T_{1/2\gamma}$ R:S ratio	0.75 (0.71–0.79; $P < 0.0001$)	0.67 (0.53–0.81; $P = 0.001$)	0.69 (0.67–0.72; $P < 0.0001$)	NSD

A dose of 250 mg *rac*-thiopental (as sodium salt) was infused intravenously over 3 min. Data are presented as mean (and 95% CIs). Pharmacokinetic values are given for *rac*-thiopental: Cl_t = mean total body clearance; $T_{1/2\gamma}$ = slow half-life; V_c = initial dilution volume; V_{ss} = total (steady-state) apparent distribution volume. Values for the R:S ratio of the pharmacokinetic values in the same experiment reflect within-animal values and are tested against a value of unity: 95% CI of the ratio that do not contain unity indicate significant enantiomeric bias.

NSD = not significantly different between acid-base treatment groups.

an unchanged total body clearance, produced a prolonged slow half-life (T1/2γ). It is reasonable to surmise that this resulted from an increased distribution into tissue of unbound nonionized drug. We did not find that acid–base derangement differentially influenced pharmacokinetics between enantiomers of either drug.

Humans with bupivacaine toxicity may be acidotic because of preexisting abnormality or to consequences of bupivacaine toxicity itself, or alkalotic from effects of resuscitation attempts. Others have found that CNS toxicity of local anesthetic drugs was increased by respiratory acidosis imposed on preexisting metabolic acid–base derangement[31] and that respiratory acidosis with moderate hypoxia increased the cardiotoxicity of bupivacaine.[7] The importance of combined hypoxia and acidosis was emphasized by another study in which neither hypoxia nor simulated respiratory acidosis enhanced local anesthetic–induced depression of guinea pig atria *in vitro*, but acidosis plus hypoxia did enhance bupivacaine-induced depression of heart rate and contractile force.[32] The aim of our study was to determine the cardiovascular and pharmacokinetic outcome resulting from changes to blood pH. The subjects were not anesthetized and ventilated because of the known confounding effect of general anesthetics on these same variables.[33] Our method of imposing acidemia represents the accumulation of plasma lactate during convulsions, with maintenance of airway and coronary blood flow, thus avoiding myocardial hypoxia. At the doses studied, cardiovascular depression was not worsened by acidemia, and this is consistent with the outcome of an *in vitro* study with imposed acidosis and lidocaine cardiotoxicity.[34] In other experiments with acidemia from electrically induced muscular tetany, combined metabolic and respiratory acidosis were found to decrease the myocardium and brain tissue:plasma distribution coefficients of bupivacaine.[28] This was reasoned to have occurred because the difference between intracellular and extracellular pH may have been less than normal (decreased extracellular and unchanged intracellular pH), and therefore, ion trapping in myocardium and brain would have been lessened. The current results show that when myocardial oxygen supply is adequate, acidemia *per se* does not alter pharmacokinetics in a way that would enhance bupivacaine toxicity.

Dosage requirements of thiopental are decreased in the presence of increased blood pH resulting from slight over ventilation.[35] This was attributed to a decreased rate of thiopental uptake into fat.[35] Previous studies on thiopental disposition in dogs found that hypercapnia-induced acidemia to pH 6.8 reduced plasma thiopental concentrations by 40%, but increasing the pH to 7.7 had no effect.[36] Acidemia was believed to have caused an increased rate of drug transfer into tissues, including the CNS.[36] However, there were no pharmacokinetic changes found in our study to support any of these observations. Thiopental activity at γ-aminobutyric acid type A receptors is weakly pH dependent with greater activity of both enantiomers found at pH 7.0 than 8.0,[37] but pharmacodynamics were not evaluated enantioselectively in the current study. Modestly enantioselective pharmacokinetics were identified for thiopental, consistent with our previous observations,[12,38] but were not sensitive to changes in acid–base status.

In conclusion, some effects of bupivacaine and thiopental were modified by changes in acid–base status, *e.g.*, the detrimental cardiovascular effects of bupivacaine were lessened by acidemia. However, such pharmacodynamic changes could not be ascribed to changes in regional or whole body pharmacokinetics. Acid–base disturbances unaccompanied by hypoxia did not significantly exacerbate the adverse cardiovascular effects of bupivacaine or thiopental at the doses studied.

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Appendix: Automatic Baseline Correction

Corrections of baseline slope (baseline linear) drift and baseline first-order curvature (log or first exponential) were effected in two stages. The first corrected for first-order curvature of the baseline, and the second corrected for slope of baseline. Higher-order corrections had to be performed in diminishing order so that the last operation performed was slope correction. We define a first-order curve as one of which the first differential is linear. A second-order curve would be one of which the differential is a first-order curve. Slope is zero-order curvature. A parabolic curve ($y = ax^2 + bx + c$) differentiates to a straight line $y = 2ax + b$; thus, a parabola by our working definition is a first-order curve.

Curvature correction was begun by finding the first differential of the data with respect to time and calculating its slope. This slope is the average second differentiation. The data were then differentiated again to find d^2y/dx^2. Dividing the average of the second differentiation by 2 produced a correction factor that was subtracted from each point during integration of the second differential. A second integration produces the original data set, but corrected for first-order curvature.

The baseline slope was corrected similarly. Finding the slope of the undifferentiated and curve-corrected data and dividing it by two provided the correction factor. On differentiation of the curvature-corrected data, we then corrected for slope by adding the correction factor to each datum at reintegration to provide a fully curvature- and slope-corrected baseline.

Only baseline data (before and after response) were used for finding the two correction factors for data normalization. The corrections were then applied to all data, both baseline and drug response. The abscissa at the time of commencement of drug delivery was set to its unprocessed value. By including the post–drug effect data in the calculation of correction factors, correction was by interpolation, rather than extrapolation, because this is a much safer method for data preservation. When correction factors were calculated from all data, rather than baseline only, the baseline correction was not as effective, although in many cases, the algorithm still improved correction of the baseline drift.