British Journal of Pharmacology (2001) 132, 649–658

www.nature.com/bjp

Direct cardiac effects of intracoronary bupivacaine, levobupivacaine and ropivacaine in the sheep

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1 The racemic local anaesthetic agent bupivacaine is widely used clinically for its long duration of action. Levobupivacaine and ropivacaine are bupivacaine enantiopure congeners, developed to improve upon the clinical safety of bupivacaine, especially the risk of fatal arrhythmogenesis.

2 In previous preclinical studies of the safety of these drugs with intravenous administration in conscious ewes over a wide dose range, we found that central nervous system (CNS) excito-toxicity reversed the cardiac depressant effects when doses approached the convulsant threshold and thus precluded accurate comparison of their cardiovascular system (CVS) effects.

3 To study CVS effects over a wide range of doses with minimal CNS and other influences, brief (3 min) infusions of bupivacaine, levobupivacaine or ropivacaine were administered into the left main coronary arteries of previously instrumented conscious ewes ($\sim$50 Kg body weight). After dose-ranging studies, the drugs were compared in a randomized, blinded, parallel group design. Equimolar doses were increased from 8 μmol ($\approx$2.5 mg) in 8 μmol increments, to either a fatal outcome or a 40 μmol ($\approx$12.5 mg) maximum.

4 All three drugs produced tachycardia, decreased myocardial contractility and stroke volume and widening of electrocardiographic QRS complexes. Thirteen of 19 animals died of ventricular fibrillation: four of six with bupivacaine (mean $\pm$ s.e.mean actual fatal dose: 21.8 $\pm$ 6.4 μmol), five of seven with levobupivacaine (22.9 $\pm$ 3.5 μmol), four of six with ropivacaine (22.9 $\pm$ 5.9 μmol). No significant differences in survival or in fatal doses between these drugs were found.

5 The findings suggest that ropivacaine, levobupivacaine and bupivacaine have similar intrinsic ability to cause direct fatal cardiac toxicity when administered by left intracoronary arterial infusion in conscious sheep and do not explain the differences between the drugs found with intravenous dosage.

British Journal of Pharmacology (2001) **132,** 649–658

Keywords: Local anaesthetics; bupivacaine; levobupivacaine; ropivacaine; coronary circulation; myocardial contractility; ventricular arrhythmias; sheep

Abbreviations: ANOVA, analysis of variance; CI, confidence interval; CO, cardiac output; dP/dt_{max}, maximum value of the first derivative of LVP; E_{max}, magnitude of peak effects; HR, heart rate; i.v., intravenous: LCA, left coronary artery; LVP, left ventricular pressure; MED, mean effect difference, SED_5 and SED_{10}, sum of the effect differences, respectively, to 5 and 10 min; PVC, premature ventricular contractions; QTc, HR corrected QT interval; R-bupivacaine or dexbupivacaine, (+)-R-bupivacaine; S-bupivacaine or levobupivacaine, (−)-S-bupivacaine; SV, stroke volume; T_{max}, time of E_{max}; VF, ventricular fibrillation

Introduction

For several decades, bupivacaine [(RS)-1-butyl-2-piperydyl-formo-2′,6′-xylidide hydrochloride] has enjoyed great clinical popularity as a local anaesthetic agent, due to its long duration of neural blockade as compared with congeners such as lignocaine and mepivacaine. However, the requirements for long duration and low toxicity do not sit well together, and numerous studies have now indicated that bupivacaine can cause serious cardiac toxicity, a primary clinical concern, with a disproportionately greater incidence than its shorter-acting congeners (Tucker & Mather, 1998).

Bupivacaine is used clinically as a racemate, but its component enantiomers demonstrate some significant pharmacological differences. Notably, S-bupivacaine (nonproprietary name, levobupivacaine) is less potent than R-bupivacaine (dexbupivacaine) in producing central nervous system (CNS) excito-toxicity, and in producing potentially fatal cardiovascular system (CVS) toxicity, especially arrhythmogenesis (Åberg, 1972; Luduena *et al*., 1972; Mather, 1991). Accordingly, levobupivacaine has been proposed as a possible replacement for bupivacaine and is currently undergoing clinical trials (Foster & Markham, 2000; Gennery *et al*., 2000). Because of its lesser toxicity compared to bupivacaine, ropivacaine, the 1-propyl homologue of levobupivacaine, has already been accepted into anaesthetic clinical practice (Markham & Faulds, 1996).

Our previous preclinical pharmacological studies were designed to determine the relative CNS and CVS effects of

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the newer enantiopure local anaesthetics compared to bupivacaine with intravenous (i.v.) administration in intact conscious sheep (Nancarrow *et al*., 1989; Rutten *et al*., 1989; Huang *et al*., 1998; Chang *et al*., 2000). This study design was used to simulate accidental i.v. injection of local anaesthetic solution during a neural blockade procedure. It was found that initial myocardial depression directly induced by these drugs was reversed with larger doses that caused CNS stimulation (convulsive behaviour), thereby preventing a clear evaluation of their direct cardiac effects as the doses became increasingly toxic. The significance of this observation is that the cardiac toxicity may derive from both direct cardiac effects and indirect CNS mediated effects (Heavner, 1986; Nancarrow *et al*., 1989; Denson *et al*., 1992; Bernards & Artu, 1993). Thus, most previous attempts to compare the cardiac effects these drugs over a wide range of doses have been restricted to *ex vivo* studies where no such interaction is possible. Although useful, such study paradigms lack anatomical and physiological fidelity and may not accurately represent the effects of the drugs *in vivo* or give insights into their mechanisms. The latter aspect if especially important if rational clinical treatment strategies for toxicity are to be developed.

In order to perform comparative studies of the direct cardiac effects of bupivacaine, levobupivacaine and ropivacaine *in vivo*, without confounding effects from the CNS, we developed and employed a novel intact, chronic and conscious sheep preparation that allowed the drugs to be administered directly to the heart, *via* the coronary arterial blood supply, in relative doses similar to those reaching the heart from i.v. administration, but without significant recirculation and potentially confounding effects from the CNS.

Methods

The study was designed to compare the direct cardiac effects of bupivacaine, levobupivacaine and ropivacaine, over a range of doses from trivial to potentially fatal, and was approved by the institutional Animal Care and Ethics Committee.

Surgical procedures and experimental protocol

Non-pregnant Merino/Border Leicester first cross ewes (40–55 kg) were used. Under halothane anaesthesia, a left thoracotomy was performed to implant an active redirection transit time flow probe (21 mm Triton 200-306-M; Triton Technology Inc, San Diego, CA, U.S.A.) around the pulmonary artery for cadiac output (CO) measurement. A spinal catheter (22 G Spinocath, B Braun Melsengen AG, Melsengen, Germany) was placed retrograde in the left anterior descending coronary artery (paraconal artery in the sheep) and directed into the left coronary artery (LCA) to 1 mm proximal to the external LCA bifurcation as validated by fluoroscopy (Figure 1). A pressure transducer catheter (Millar 3 Fr SPR-52, Millar Instruments Inc., Houston, TX, U.S.A.) was placed into the left ventricle through the free wall for the measurement of left ventricular pressure (LVP). A pair of ECG wires was secured to the dorsal and ventral extremities of the fifth rib. The chest was closed layer by layer. The coronary artery catheter was flushed with glucose-heparin lock solution. Approximately 1 week later, two catheters were placed in the carotid artery and advanced into the aortic arch for measurement of MAP and for arterial blood sampling. A pacing lead was placed in the right atrium for intra-atrial ECG recording. Five to 7 days were allowed for recovery before the first pharmacological study. The sheep were maintained in metabolic crates. During the study, they were placed in a sling to prevent recumbency.

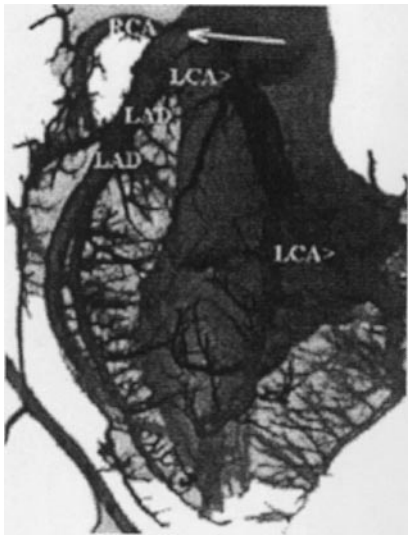

Figure 1 Photograph of a cast of the coronary anatomy of the sheep showing the left circumflex (LCA), the anterior descending (paraconal) (LAD) and the right coronary arterial vessels as the project from the aorta of the sheep. The placement of the catheter tip was at the bifurcation of these vessels as shown by the arrow.

Drugs

Bupivacaine HC1 (Marcaine™, Delta West, Perth, Australia), ropivacaine HCl (Naropin™, Astra-Zeneca Pty Ltd, Sydney, Australia) and levobupivacaine HCl (Chirocaine™, Celltech-Chiroscience, Cambridge, U.K.) were administered in 15 ml of 0.9% saline solution and were used in equimolar concentrations to overcome differences in commercially prepared drug concentrations as to salt *versus* base.

Dose-finding study

Initial doses, projected from previously used i.v. doses (Mather *et al*., 1998; Huang *et al*., 1998) and the estimated coronary fraction of cardiac output, were tested in five animals using a cross-over design with bupivacaine HCl, levobupivacaine HCl or ropivacaine HCl, infused directly into the LCA over 3 min. Data obtained after drug administration were compared to those obtained in the 5 min immediately preceding drug administration. Doses started at 8 μmol ($\approx$2.5 mg) and were increased in 8 μmol increments on consecutive study days to a maximum of 40 μmol ($\approx$12.5 mg) or fatality. A random drug order, with 24 h for washout between studies, was used at the 8 and 16 μmol doses, but bupivacaine was administered last at larger doses to minimize the probability of a premature fatality.

Systematic parallel group study

Twenty-four animals were allocated randomly to parallel groups to receive one of the three drugs in a dosage scheme selected after the dose-finding study. The drugs, in doses of 8, 16, 24, 32 or 40 μmol, were infused directly into the LCA over 3 min; the drug solutions were prepared coded and the code remained unbroken until the data analyses were completed.

The experiments started with a 5 min baseline measurement, followed by a 3 min intracoronary infusion of 0.9% saline (15 ml) as a vehicle control; 30 min later, intracoronary administration of the test drug (15 ml) was performed and the data recording was continued for 60 min (Figure 2). Drug doses were increased in 8 μmol increments on consecutive study days to a maximum of 40 μmol or fatality. Nineteen sets of data (bupivacaine, $n=6$; levobupivacaine, $n=7$ and ropivacaine, $n=6$) were obtained for final analyses. At conclusion of the set of experiments, surviving animals were anaesthetized with pentobarbitone and blue food dye was injected into their coronary arterial infusion cannulae to visualize the areas perfused. The animals were then euthanased and a post-mortem was performed for probe retrieval and evaluation of the cannula tip position. If the animals died from test drug, samples of cardiac muscle were taken from right atrium and ventricle, left atrium and ventricle and intraventricular septum for drug concentration analyses.

Data acquisition and processing

Analogue signals, consisting of ECG, MAP, CO, LVP and first derivative of LVP (dP/dt) data, were acquired at 256 Hz by a physiological monitoring system (System 6, Triton Technology Inc.,) and digitally converted (MP100, Biopac Systems Inc, Santa Barbara, CA, U.S.A.) and captured using a personal computer. Derived data consisted of heart rate (HR), stroke volume ($SV=CO/HR$), the maximum positive value of dP/dt (dP/dt_{max}) and the ECG parameters PR interval, QRS width, RR interval, QT interval and corrected QT interval from the formula $QTc=QT/\sqrt{(RR)}$.

The scheme of the experiments is shown in Figure 2. Baseline data, defined during the 5 min prior to saline and drug, were averaged and assigned values of 100% for comparison with the subsequent values from the respective saline and drug infusion periods. Haemodynamic data were divided into 20 s epochs and the epoch averages were analysed in Microsoft Excel 5.0. Five consecutive ECG complexes were examined every 1 min during the baseline period; every 20 s for the first 5 min after the commencement of saline and drug infusions, and every 5 min until the end of saline or drug period. Cardiac arrhythmias were identified, recorded and expressed as the ratio of the number of affected animals to all treated animals for any drug dose.

The numbers of the original cohort of animals that survived after all doses up to that dose were determined ('survivors'). The doses infused to the time of cardiac death were determined for comparison between drugs ('non-survivors'). The time of death was defined by the earlier of complete (sudden) cessation of CO or by the inflection point on an inexorable trend as MAP decreased towards estimated mean circulatory pressure.

Arterial blood samples were collected immediately before drug infusion, then at 1, 2, 3, 4, 5, 10 and 30 min later as measured of the drug concentrations recirculated to other tissues. These, and samples of heart tissue taken when the animals died from the test drugs, were analysed for drug concentration by chiral HPLC (Gu *et al*., 1998).

Data analysis

Data ('effect differences'), consisting of the differences between values in the saline (control) or drug periods and their respective baseline periods (B1 and B2, Figure 2) were expressed as percentages of the baseline values. Data from the survivors were analysed for the magnitude of peak effects (E_{max}) and time of E_{max} (T_{max}). In addition, the sum of the effect differences, to 10 min (SED_{10}) for haemodynamic data

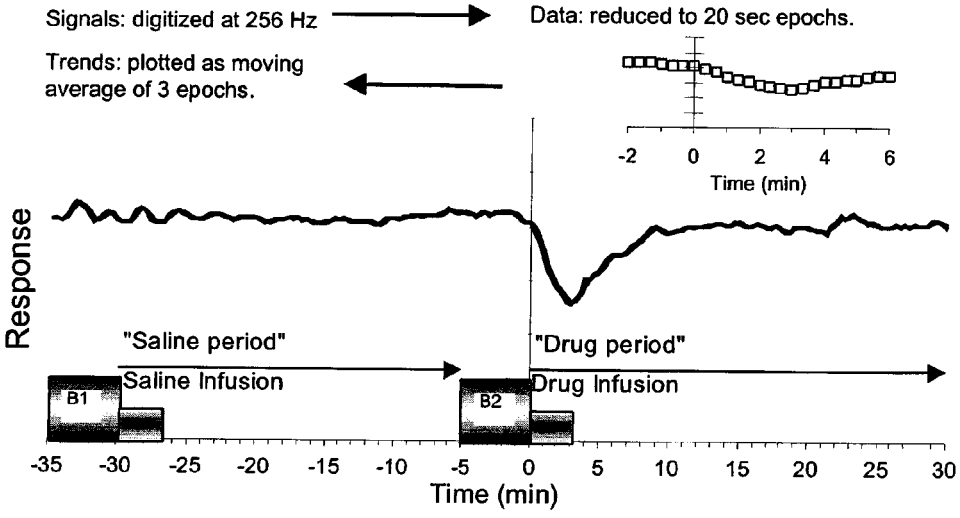

Figure 2 Schematic representation of the study design for the parallel groups. The 5 min baseline periods (stippled bars B1 and B2) were used to normalize the effects of 3 min saline (vehicle control) and drug infusions, respectively (shaded bars Saline Infusion and Drug Infusion).

and to 5 min (SED_5) for ECG data, were determined to capture differences in magnitude and immediate time course: these data are analogous to areas under the curves (Mathews *et al*., 1990).

Derived variable data from survivors were analysed by fitting random effects linear models (Lindsey, 1999) using the cross-sectional time series regression facility of the statistical software Stata version 6 (Stata Corporation, College Station, TX, U.S.A.). The random effects model allowed the fitting of dose-response curves for all drug doses simultaneously while making allowance for random variation in level of response among animals. Dose-response relationships were tested for linearity by adding a quadratic term in dose, and for parallelism by adding terms describing interactions between the linear dose trend and drug. If a significant difference among dose-response curves of the three drugs was found, curves of the drugs were compared pairwise using Wald tests.

Possible differences in mortality rate among the three drugs were examined by Cox proportional hazards regression (Hosmer & Lemeshow, 1999). Explanatory variables consisted of the dose of drug given up to the time of death (or maximum dose given for survivors) and dummy variables identifying the drug given. If there was a significant difference in mortality among the three drugs, they were compared pairwise.

The null hypothesis of equality of the three drugs was retained. A significance criterion of $P<0.05$ was taken as weak evidence for rejection of the null hypothesis, a significance criterion of $P<0.01$ was taken as strong evidence. All tests were two-tailed. Data are expressed as mean ($\pm$s.e.mean) unless otherwise specified.

Results

Dose-finding study

Four animals died of bupivacaine infusion: one at 16 μmol; two at 24 μmol (having previously survived 16 μmol ropivacaine and levobupivacaine); and one at 40 μmol (having previously survived 40 μmol ropivacaine and levobupivacaine) One animal died of 24 μmol levobupivacaine infusion having survived 16 μmol of all three drugs. Decreased LV dP/dt_{max} and SV, accompanied by increased HR were found, with maximum changes at 3–5 min and recovery to baseline values by 10 min. Deaths were due to sudden-onset ventricular fibrillation (VF).

Systematic parallel group study

Nineteen animals gave the final data set, six received bypivacaine, seven received levobupivacine and six received ropivacaine. There were no differences between the mean weights of the animals receiving each of the test articles (one-way ANOVA, $P=0.43$). Three of the remaining animals were found to have variant coronary artery anatomy not suitable for cannulation and were euthanased under anaesthesia; two animals were found at post-mortem to have had incorrect catheter placement. The numbers of animals treated/survived with the 8, 16, 24, 32 and 40 μmol doses differed due to attrition and were, respectively, bupivacaine: 6/6, 6/5, 5/3, 3/3 and 3/2; levobupivacaine: 7/7, 7/7, 7/3, 3/2 and 2/2; ropivacaine: 6/6, 6/6, 6/4, 4/3 and 3/2.

No significant responses to saline infusion were observed in E_{max} of any parameter in any animal and further analysis of the 'saline period' data was not performed.

For the data of the 'drug period', in all cases where a drug dose-response relationship was found, the quadratic term was not significant and the model was collapsed to the (simpler) linear form. No evidence of significant differences in slopes of the dose-response curves between drugs was found for any parameter.

There were no significant differences between the three drugs in either the frequency of survival or of fatal doses. The actual doses infused to fatality were: bupivacaine, 21.8 ($\pm$6.4) μmol ($n=4$ of 6 animals), levobupivacaine, 22.9 ($\pm$3.5) μmol ($n=5$ of 7 animals) and ropivacaine, 22.9 ($\pm$5.9) μmol ($n=4$ of 6 animals). The hazard ratio from Cox regression was 1.1 (95% CI 0.3–4.4) for bupivacaine : ropivacaine and 1.0 (95% CI 0.3–3.6) for levobupivacaine : ropivacaine: these values were not significantly different to unity. Median lethal doses from Kaplan–Meier analysis were bupivacaine 18.7 μmol, levobupivacaine 24.1 μmol and ropivacaine 24.1 μmol. Death, when occurring from each drug, was due to sudden-onset VF (Figure 3).

Effects in survivors

Data are shown in Figures 4 and 5. When changes occurred from drug infusion, a T_{max} of 3–5 min was observed with no significant differences between drugs or doses.

Haemodynamic findings All three drugs produced significant decreases in LV dP/dt_{max} and SV. Regarding LV dP/dt_{max}, ropivacaine was less potent (judged by E_{max}) than bupivacaine ($P=0.011$) or levobupivacaine ($P=0.002$), which were not significantly different. Judged by SED_{10} and MED, the duration of effect of ropivacaine was briefer than bupivacaine ($P=0.02$), and lasted longer than levobupivacaine ($P=0.0001$). Regarding SV, ropivacaine was less potent than bupivacaine ($P=0.014$) and levobupivacaine ($P=0.001$); ropivacaine was also shorter acting than levobupivacaine ($P=0.002$). All three drugs produced tachycardia; bupivacaine was more potent and longer acting than levobupivacaine (respectively, $P=0.008$ and $P=0.029$), and levobupivacaine was more potent than ropivacaine (respectively, $P=0.017$ and $P=0.006$). Neither CO nor MAP was significantly altered by any of the drugs.

Electrocardiographic findings Neither PR interval nor QTc were significantly altered by any drug. QRS width was increased, and the potency and duration of bupivacaine was greater than that of ropivacaine (respectively, $P=0.014$ and $P=0.010$); there were no significant differences between the other drug pairs. Ventricular arrhythmias occurred frequently with all drugs. Premature ventricular contractions (PVCs) were the most common arrhythmia but there were no significant differences in frequency of PVCs between drugs, nor in dose-response relationships.

Effects in non-survivors

Relative drug effects at the fatal doses can be gleaned from the data for the epoch before the onset of fatal VF. Interpretation needs caution because of the difficulty in

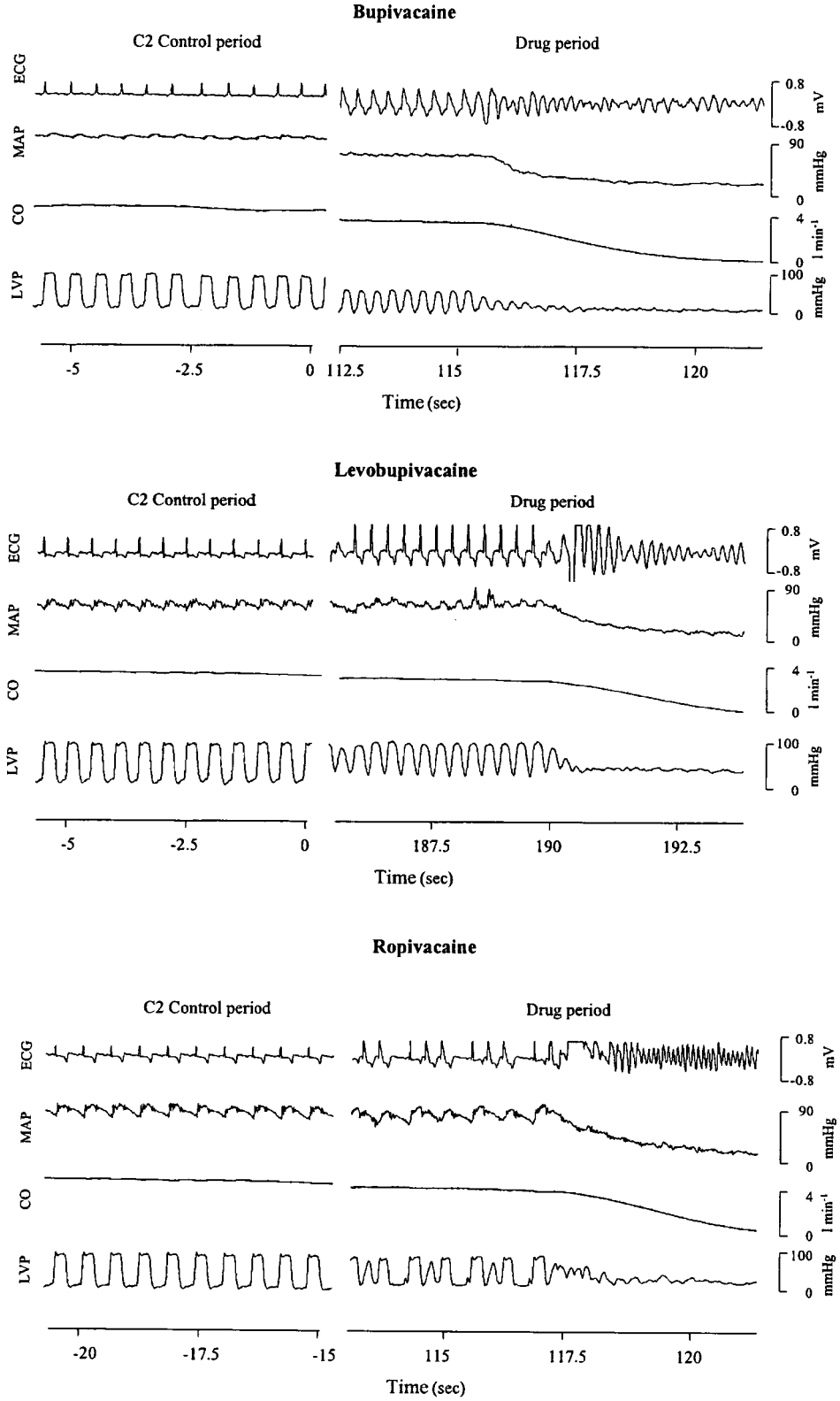

Figure 3 Composite traces of internal right atrial ECG, MAP, CO and LVP immediately before (left panels) and at various times after the infusion of bupivacaine (16 μmol), levobupivacaine (24 μmol), or ropivacaine (16 μmol) into the left coronary arteries of individual sheep (right panels). In each case the assigned doses were to be 24 μmol infused over 180 s but the animals receiving the infusions of bupivacaine and ropivacaine died of malignant cardiac arrhythmias-ventricular fibrillation at the times shown and before the full infusion could be given.

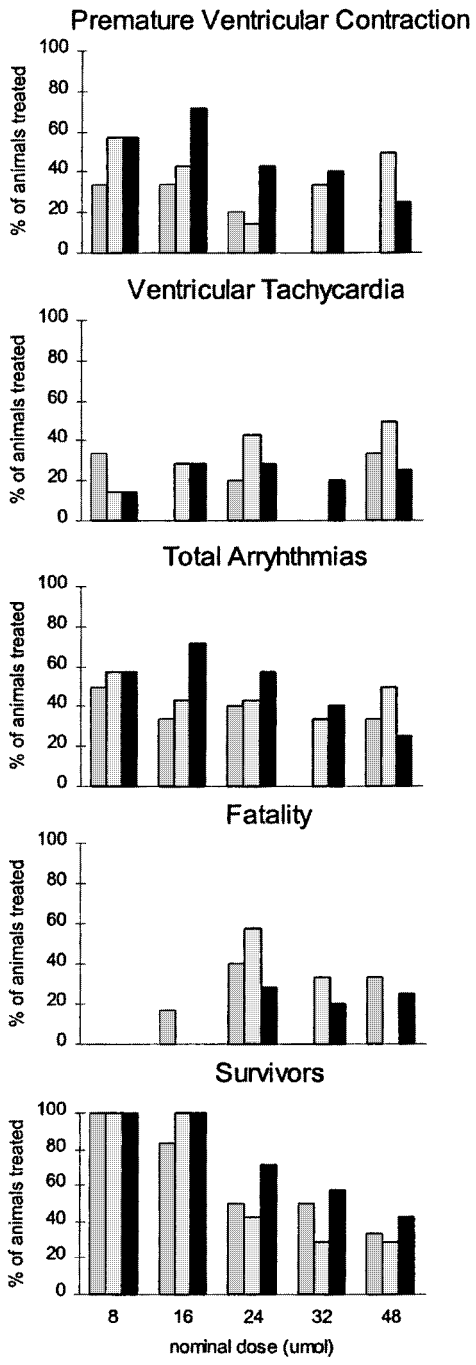

Figure 4 The effect of intracoronary arterial infusion of bupivacaine (grey), levobupivacaine (white) and ropivacaine (black) on the occurrence of ventricular and total numbers of all arrhythmias (% affected animals/treated animals for that dose). The numbers of animals treated/survived with the 8, 16, 24, 32 or 40 μmol doses were, respectively, bupivacaine: 6/6, 6/5, 5/3, 3/3, and 3/2; levobupivacaine: 7/7, 7/7, 7/3, 3/2, and 2/2; ropivacaine: 6/6, 6/6, 6/4, 4/3, and 3/2.

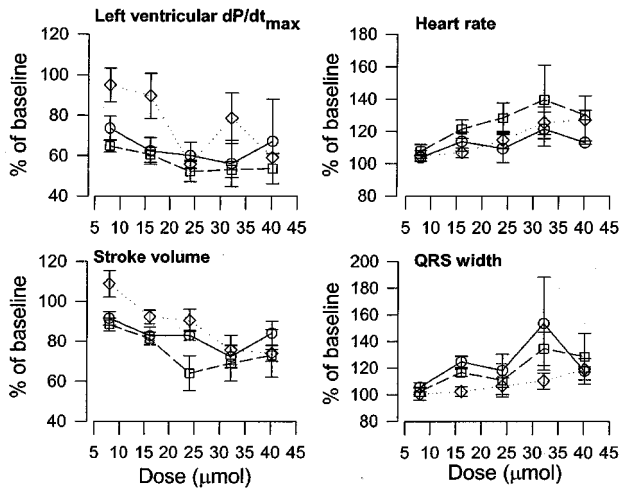

Figure 5 Maximum effects (E_{max}) of bupivacaine, levobupivacaine and ropivacaine on haemodynamic changes and cardiac electrical activity in survivors plotted as a function of dose. The numbers of animals with the 8, 16, 24, 32 or 40 μmol doses were, respectively, bupivacaine: 6, 5, 3, 3 and 2; levopubivacaine: 7, 7, 3, 2 and 2; ropivacaine: 6, 6, 4, 2 and 2.

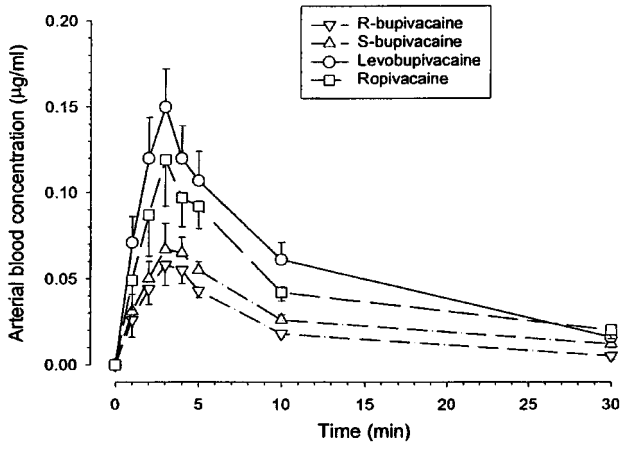

Figure 6 Time course of the aortic blood concentrations of bupivacaine, levobupivacaine and ropivacaine following intracoronary infusion of the 8 μmol does in all sheep. Mean and s.e.mean are shown. The concentrations of R- and S-bupivacaine from administration of bupivacaine are shown separately. The numbers of animals were, respectively, bupivacaine: 6; levobupivacaine: 7; ropivacaine: 6.

assigning accurate values when the animals were deteriorating rapidly over different times after commencement of drug infusion. One animal receiving ropivacaine died at 7 min compared to 1–4 min for all others.

Haemodynamic findings LV dP/dt_{max} and SV decreased markedly whereas HR increased; but there were no systematic differences between the three drugs. MAP and CO were not regularly altered by any of the drugs.

Electrocardiographic findings PR interval was not demonstrably altered; QTc increased above baseline with bupivacaine ($112 \pm 4\%$, $P = 0.025$) but there were no significant differences between drugs. QRS width tended to increase with time for all drugs; the changes at 2 min were significant (i.e. > baseline) for bupivacaine $137 \pm 6\%$ of baseline ($P = 0.01$) but not for levobupivacaine $109 \pm 5\%$ of baseline ($P = 0.13$, ns) or ropivacaine 103 ± 5 of baseline ($P = 0.54$, ns). The difference in magnitude of QRS width at 2 min between drugs was significant ($P = 0.005$, one-way ANOVA).

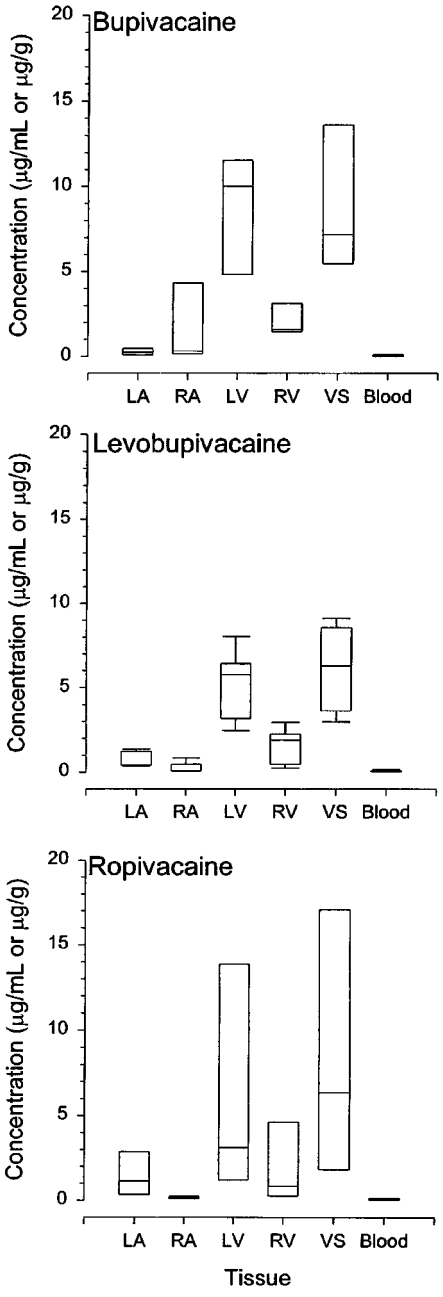

Figure 7 Box plots of the concentrations of bupivacaine, levobupivacaine and ropivacaine in myocardial tissues and aortic blood following fatal intracoronary infusion in 'nonsurvivor' sheep (bupivacaine: $n=3$; levobupivacaine: $n=5$; ropivacaine: $n=4$). LA = left atrium, RA = right atrium, LV = left ventricle, RV = right ventricle, VS = ventricular septum, Blood = aortic blood.

Blood and tissue drug concentrations

The aortic drug concentrations in both survivors and non-survivors were not at levels expected to cause significant CNS effects. The time course of the blood drug concentrations is shown for the lowest dose (Figure 6). Maximal concentrations occurred at 3–4 min and were essentially proportional to dose. After doses of bupivacaine, blood concentrations of S-bupivacaine slightly but consistently exceeded those of R-bupivacaine. However, the summed concentrations of the two enantiomers were similar to those of levobupivacaine and ropivacaine. Tissue analysis of the non-survivors (Figure 7) indicated that drug concentrations in left ventricular tissue and septum tended to be the highest, and that there were no differences between drugs in their tissue uptake.

Discussion

The aim of this study was to compare the direct cardiac effects of bupivacaine, levobupivacaine and ropivacaine with minimal influence from their CNS and other effects. The study design required incorporation of several issues that had not previously been included together in any other attempt to study this, or analogous, problem.

Our chronic preparation allowed the study of individual dose-acute response relationships in conscious animals with intact central neural control mechanisms, thereby allowing reasonable comparison with previous studies using i.v. drug administration (Nancarrow *et al*., 1989; Rutten *et al*., 1989; Huang *et al*., 1998). This preparation offers advantages over both acute *ex vivo* spontaneously beating heart preparations (e.g. Pitkanen *et al*., 1992) where no such control mechanisms remain intact, and acute *in vivo* preparations in which the resultant cardiovascular effects may be influenced by the anaesthetics or other medications used (Bertrix *et al*., 1991).

Intracoronary infusion allowed similar amounts of drug to be delivered to the left heart as from i.v. infusions, but without sufficient recirculation of drug to cause significant CNS and other effects. It was found that neither the blood drug concentrations nor the tissue drug concentrations showed any tendency to differ markedly, between drugs and the aortic blood drug concentrations of each of the drugs were insignificant with respect to producing significant systemic effects, although enantiomeric differences were noted for bupivacaine, as expected from previous studies (Mather *et al*., 1994). Although intracoronary infusion has been used by others to study the direct cardiac effects of local anaesthetics (e.g. Reiz *et al*., 1989; Buffington, 1989; Fujita *et al*., 1996; Morrison *et al*., 2000), none has used a chronic intact conscious preparation nor verified their assumptions with pharmacokinetic methods. From coronary vasculature erosion casts (Figure 1) it was possible to determine and fix the correct placement of the catheter tip; this was made retrograde so that drug infused could be thoroughly mixed in the afferent blood to preclude streaming. The regions found to be mainly enriched with the drugs were the left ventricle and septum (Figure 7), consistent with predictions from the vascular casts.

Intracoronary infusion of saline indicated that there was no effect from the drug solution vehicle. A random effects linear model allowed examination of the dose-response relationships and differences among drugs in the presence of variation in responsiveness among animals and missing data due to attrition at the higher doses. While the non-random pattern of the missing data creates a potential for bias in assessing dose-response relationships, its impact on comparisons among drugs is likely to be minimal as mortality was similar for the three drugs.

All of the drugs produced decreased LV dP/dt_{max}, decreased SV, increased HR, widened QRS complexes and

fatal cardiac arrhythmias over the same dose range. The effects tended to be least, overall, for ropivacaine. It is probable that the changes in HR and SV were secondary to changes in myocardial contractility and that the chronotropic effect was due to baroreceptor reflex activity acting by increasing the HR to maintain MAP in the face of diminished myocardial contractility (Chang *et al*., 1994). It is probable that arrhythmias were due to re-entrant phenomena caused by drug-induced conduction disorders (de la Coussaye *et al*., 1992). Death, when it occurred with all three drugs, was due to VF, with no distinction between drugs.

Others have recently reported that intracoronary injection in pigs of bupivacaine *in vivo* would more potently produce QRS widening and fatal VF than levobupivacaine or ropivacaine (Morrison *et al*., 2000); similar findings have been made with the isolated heart preparations (Mazoit *et al*., 1993; 2000). Although, in studies in the pig, the fatal drug doses were similar to those in the sheep, various methodological differences, apart from species, may have influenced the outcome. The pigs were acute anaesthetized (pentobarbitone) preparations, dosed repeatedly ($\sim$5–10 min apart, upon recovery of ECG parameters) until fatal, whereas the sheep were conscious, chronic preparations and administered a single dose once daily. The pigs had their catheter tips in the proximal left anterior descending artery just after bifurcation of the main stem, whereas those in the sheep were at the bifurcation. A major difference was, however, that the cumulative fatal doses used in the pigs may have been large enough to have produced additional systemic effect; this is hardly likely from the single doses and measured resultant drug arterial blood concentrations in the sheep.

Previous studies with i.v. administration found bupivacaine to be clearly more arrhythmogenic, and thus more grossly toxic, than either levobupivacaine or ropivacaine (Gennery *et al*., 2000). The discrepancy between the present and previous studies may be due to any of the following reasons. The greater arrhythmogenesis of i.v. bupivacaine may result from its more potent effects on the CNS, where the convulsant doses are in the rank order ropivacaine > levobupivacaine > bupivacaine (Gennery *et al*., 2000). This possibility is currently being tested using site directed drug delivery to brain with minimal recirculation to heart (work in progress). The right coronary arteries were not cannulated, hence it is possible that the relative lack of drug delivery to the SA and AV nodes, but with preserved sinus rhythm and relative drug enrichment at the conducting bundles, could have altered the difference in arrhythmogenic potency between the test drugs in this study (Yoshida, 1981).

Local anaesthetics are believed to depress LV dP/dt_{max} directly, essentially in proportion to their local anaesthetic activity. Hence it is not surprising that no real differences between bupivacaine and levobupivacaine in their effects on LV dP/dt_{max} were found since these drugs are of similar intrinsic local anaesthetic (Kanai *et al*., 2000) and clinical potency (Lyons *et al*., 1998); ropivacaine had a lesser effect and is less potent as a local anaesthetic agent (Polley *et al*., 1999). Similar results have been found in *ex vivo* isolated heart and cardiac myocyte preparations (Graf *et al*., 1997; Harding *et al*., 1998). Other investigations have found differences between the bupivacaine enantiomers in their blockade of selective voltage-gated ion channels in nerves and, notably, in the heart.

Local anaesthetics block Na^+ channels in a complex voltage- and frequency-dependent manner where they have a higher affinity for the channels in open or inactivated form than for the resting form. In amphibian peripheral nerves, relatively weak enantioselectivity in the potency ratio was found (R-/S-bupivacaine = $\sim$1.5) for tonic block of axonal Na^+ currents (Nau *et al*., 1999) by the bupivacaine enantiomers: similar enantioselectivity was found for blockade of propagation of the compound action potential (Lee-Son *et al*., 1992). Greater potency and enantioselectivity, however, was found for an axonal flicker K^+ channel (R-/S-bupivacaine = $\sim$73) (Nau *et al*., 1999). Enantioselectivity was also found in the kinetics of association and dissociation at the amphibian peripheral nerve flicker K^+ channel where the dissociation rate of dexbupivacaine was much slower than that of levobupivacaine, such that their ratio was similar to the potency ratio (R-/S-bupivacaine = $\sim$64). In other studies, enantioselectivity for displacement of labelled-batrachotoxin from rat brain synaptosomal Na^+ channels indicated an intermediate potency ratio (R/S = $\sim$3) with a weaker enantioselectivity in the potency for tonic inhibition of Na^+ current being R/S = $\sim$1.3 (Vladimirov *et al*., 2000).

Blockade of Na^+, K^+ and Ca^{2+} channels in the heart by bupivacaine-like drugs may all contribute to the development of fatal ventricular arrhythmias. In guinea-pig ventricular myocytes, dexbupivacaine produced a faster, more potent and slower recovering block of the inactivated Na^+ channels than levobupivacaine (Vanhoutte *et al*., 1991; Valenzuela *et al*., 1995a). A more recent study of the human heart hH1 Na^+ channels suggested a relatively weak enantioselectivity of block of the inactivated sodium channels (R-/S-bupivacaine = $\sim$1.5), with a similar enantioselectivity in the rate of recovery, but with no difference in the blockade of the resting channels between enantiomers (Nau *et al*., 2000). Thus a common finding is that dissociation of levobupivacaine from neural and cardiac Na^+ channels exceeds that of dexbupivacaine. Blockade of K^+ channels by these drugs can prolong action potential and further exacerbate Na^+ channel block. Data from the study of the effect of the bupivacaine enantiomers on the cloned human cardiac K^+ channels (hKv1.5) showed that levobupivacaine was less potent than dexbupivacaine to block the channels (Valenzuela *et al*., 1995b). Blockade of cardiac K^+ channels may be an important component in bupivacaine cardiotoxicity (Boban *et al*., 1993). At relatively higher concentration, bupivacaine may also interact with dihydropyridine-sensitive Ca^{2+} channels and slow the phase 4 depolarization of pacemaker cells during diastole (Hogan, 1996; Rossner & Freese, 1997; McCaslin & Butterworth, 2000), although other studies with the enantiomers of ropivacaine showed no significant enantioselectivity (Hirota *et al*., 1997).

Differences between drugs/enantiomers in their neural and cardiac ion channel blockade derive mainly from their propensity for producing frequency-dependent blockade with the faster and slower, respectively, rates of depolarization of neural and cardiac excitable tissues (Strichartz, 1998). Differences between drugs/enantiomers in producing cardiac effects, however, are more complex because of their dual effects on cardiac myocytes and conducting apparatus. Although the data from the above-mentioned *in vitro* studies suggest that dexbupivacaine is more potent than levobupivacaine in the blockade of cardiac Na^+ and K^+ channels, no

difference in the fatal arrhythmogenesis between bupivacaine and levobupivacaine was found in the current *in vivo* study. The reason for this discrepancy is still not clear. The current experimental design only allowed the drugs to be infused into the left ventricles and the part of the interventricular septum. The supraventricular section of the myocardial conduction system, including the SA and AV nodes and the bundle of His (at least partly) which is more important in the development of AV block and ventricular arrhythmias, received only a small fraction of the dose and a negligible fraction from recirculation. The bupivacaine enantiomers may have differential effects on the ion channels regulating cardiac conducting and inotropic effects in the ventricles. This view is consistent with the results from their study in the isolated perfused guinea-pig heart (Langendorff preparation) (Graf *et al*., 1997). In this preparation, dexbupivacaine significantly prolonged the AV conduction and produced AV dissociation compared to the racemate and levobupivacaine in all concentrations, whereas no differences were found between the enantiomers in their cardiac depressant effects. This is also consistent with the stereoselectivity of the arrhythmogenic effects between bupivacaine and levobupivacaine found previously with i.v. administration in our sheep preparation, in which all myocardial conducting tissue was exposed to the same inflowing drug arterial concentrations and producing similar atrial and ventricular tissue concentrations (Huang *et al*., 1998; Chang *et al*., 2000).

The mechanism underlying the negative inotropic effects of the local anaesthetics is more complex. It has been suggested that the negative inotropic effect is due to decreases in Ca^{2+} release from sarcoplasmic reticulum (Lynch, 1986). However, at higher concentration, modulation of cell membrane Ca^{2+} channels by local anaesthetic agents, which slowed inward Ca^{2+} current, may also play a role (Sanchez-Chapula, 1988). Furthermore, accumulated evidence suggests that local anaesthetic agents may interact with cellular energy metabolism by decreasing mitochondrial adenosine triphosphate synthesis (Sztark *et al*., 1993; 1998) and by inhibiting the basal and adrenaline-stimulated cyclic AMP production (Butterworth *et al*., 1993). All of these may contribute to local anaesthetic-induced myocardial depression. To date, few studies have explored enantioselective differences in the above effects. However, a recent study using the rat heart isolated mitochondria demonstrated no differences between the bupivacaine enantiomers in the effects of the inhibition of complex I activity and uncoupling of oxidative phosphorylation (Sztark *et al*., 2000). This may, at least partly, explain the similarity in cardiac depressant effect found in the current study.

It is concluded that ropivacaine, levobupivacaine and bupivacaine have similar ability to cause direct fatal cardiac toxicity when administered by left intracoronary arterial infusion in conscious sheep. Although ropivacaine produced the least myocardial depression and conduction changes, it also has a lower potency for producing neural blockade compared to either levobupivacaine or bupivacaine, so that its intrinsic cardiac toxicity at equi-anaesthetic doses may not be commensurately less. Quantitative differences in CVS effects between the drugs found with i.v. dosage may, in part, be a consequence of their different intrinsic CNS excitotoxicity where differences in pro-convulsant potency are known (Gennery *et al*., 2000).

The authors are pleased to acknowledge technical assistance by Dr L. Gelgor, Ms K. Wilson, Mr R. Kearns, Ms S. Gu, Ms J. Young and Ms B. Fryirs. The authors acknowledge financial support from the National Health and Medical Research Council of Australia and from Chiroscience R&D Ltd., Cambridge, U.K.

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(Received September 4, 2000
Revised November 9, 2000
Accepted November 17, 2000)