Determination of Bupivacaine in Human Fetal and Neonatal Blood Samples by Quantitative Single Ion Monitoring†

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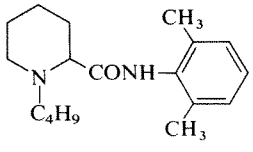
The blood levels of bupivacaine administered epidurally during labour have been determined in mother, fetus and the newborn infant by quantitative single ion monitoring, following gas chromatographic separation. Concentrations were determined from the height of the peak obtained at *m/e* 140 derived from bupivacaine compared with that at *m/e* 154 derived from the internal standard, 1-*n*-pentyl-2-(2′,6′-xylylcarbamoyl)piperidine. Results from six mothers and their infants showed that the drug passed rapidly from the maternal circulation to the infant. The plasma half-life of the drug in the newborn infant was 18 h compared with 1.25 h for the adult.

INTRODUCTION

Bupavacaine (1-*n*-butyl-2-(2′,6′)xylylcarbamoyl)-piperidine, **1**) is a long-acting local anaesthetic drug, which is at present the drug of choice for epidural anaesthesia, especially during childbirth.



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There are conflicting reports in the literature as to the possible adverse effects of bupivacaine on the fetus and/or mother when given during labour by the epidural route. Some authors[1] found no adverse effects on the fetus and neonate, while others[2,3] have reported abnormal heart rate patterns in a significant number of fetuses. Behavioural changes in babies born after epidural block with bupivacaine have also been observed.[4] One possible origin of these effects on the fetus and neonate is in the penetration of the drug across the placenta thereby having a direct effect on the infant, and to assess this we have determined the levels of bupivacaine in maternal and fetal blood during labour and in the neonate.

Bupivacaine in maternal blood was assayed by gas-liquid chromatography, but the method was not sufficiently sensitive to measure low levels of the drug in the small samples (20–100 μl) of blood obtained from the fetus and neonate. Berlin *et al.*[5] have reported a g.c. technique for the determination of bupivacaine in 200 μl samples of blood, but we have found this unreliable for present purposes. Accordingly, a quantitative method using gas chromatography mass spectrometry with single ion monitoring was developed to measure bupivacaine in these samples. This communication gives details of the methods used to measure bupivacaine and their sensitivity, and presents preliminary results obtained from six mothers and their infants.

† Abbreviation: pentyl PPX = 1-*n*-pentyl-2-(2′,6′-xylylcarbamoyl)-piperidine hydrochloride.

EXPERIMENTAL

Materials

Bupivacaine hydrochloride (m.p. 248 °C) was the gift of Duncan, Flockhart & Co., London, E2. 2-(2′6′-Xylylcarbamoyl)piperidine hydrochloride (m.p. 232 °C) was the gift of AB Bofors, Bofors, Sweden. Other compounds were obtained from commercial sources and purified before use where appropriate.

1-*n*-pentyl-2-(2′,6′-xylylcarbamoyl)-piperidine hydrochloride (Pentyl PPX). The pentyl analogue of bupivacaine was synthesized by a modification of a method for the synthesis of bupivacaine.[6] 2-(2′6′-Xylylcarbamoyl)-piperidine hydrochloride (2.32 g) was dissolved in water (100 ml), the solution adjusted to pH 14 with 10 M-NaOH and the free base so liberated extracted into ether (3 × 50 ml). The ether extracts were bulked, dried over anhydrous Na_2SO_4, filtered and the ether removed on a rotary evaporator. The residue was dissolved in pentan-1-ol (30 ml), potassium carbonate (1 g) and 1-bromopentane (1.3 g) added and the whole refluxed with stirring for 24 h. The reaction mixture was

then filtered, the precipitate washed with pentan-1-ol (10 ml) and the solvents removed on a rotary evaporator. The residue was dissolved in M-HCl (50 ml) adjusted to pH 14 with 10 M-NaOH and extracted with ether (3×50 ml). The bulked ethereal extracts were dried over anhydrous Na_2SO_4 and concentrated to *c.* 15 ml on a rotary evaporator. This solution was treated with a solution of HCl gas in dry ether (2.5 M) whereby white crystals of 1-*n*-pentyl-2-(2′,6′-xylylcarbamoyl)-piperidine hydrochloride precipitated (m.p. 221 °C). G.c. of this material showed the presence of approximately 3% of 2-(2′,6′-xylylcarbamoyl)-piperidine, and therefore the product was recrystallized twice from methanol+acetone, to a final m.p. of 229 °C. Yield 1.68 g (80%).

Subjects, drug administration and sample collection

Six obstetrically normal mothers in uncomplicated labour were studied, lumbar epidural anaesthesia being established with a dose of 35±3.2 mg (mean±s.e.) of bupivacaine. Samples (3–4 ml) of maternal venous blood were obtained through a cannula in the dorsum of the hand at 0, 10, 20, 30, 45, 60, 75 and 90 min after the epidural injection. Fetal capillary blood (20–100 μl) was obtained by puncture of the scalp at 10, 20, 30, 45, 60, 75 and 90 min after the administration of bupivacaine to the mother. Further epidural injections of bupivacaine (15–30 mg) were administered as requested by the mother during labour. Immediately after birth, a maternal blood sample was withdrawn and samples (3–4 ml) of umbilical cord venous and arterial blood obtained. Capillary blood samples were obtained from the warmed heel of the neonate at 2, 6 and 24 h after delivery. All blood samples were placed in heparinized containers and stored at −20 °C prior to analysis.

Gas chromatography

An F & M Model 402 gas chromatograph (Hewlett-Packard Ltd) equipped with flame ionization detection was used. The column was of glass, 1.52 m×3 mm i.d. and packed with 3% OV-1 on AW-DMCS treated Chromosorb G, 100/120 mesh. Operating conditions were: oven temperature, 220 °C; detector temperature, 260 °C; injector temperature 240 °C; nitrogen flow-rate 60 ml min^{-1}; hydrogen 30 ml min^{-1}; and air 300 ml min^{-1}. The column was conditioned prior to use and silanized *in situ* with bis(trimethylsilyl)acetamide (Pierce Chemical Co.) Under these conditions, the retention times of bupivacaine and pentyl PPX were 3.1 and 4 min, respectively.

Gas chromatography mass spectrometry

A Varian MAT 311A mass spectrometer was used, coupled with a Varian Aerograph 1400 gas-liquid chromatograph through a Biemann-Watson two stage separator. The column was glass, 2 m long (3 mm i.d.) packed with 3% SE30 on AW-DMCS treated Chromosorb W. The temperatures were: g.c. injection port, 290 °C; g.c. oven, 280 °C; m.s. line of sight inlet 220 °C; He separator, 265 °C; ion source, 250 °C. The helium flow rate was 45 ml min^{-1}. The electron energy was 88 eV, ionizing electron current 2000 μA, ion accelerating voltage 3 kV, and the electron multiplier voltage 2.2 kV. Under these conditions the retention times of bupivacaine and pentyl PPX were 3.6 and 4.5 min respectively, as shown by the total ion monitor. For g.c.m.s.s.i.m. the m.s. was focused on *m/e* 140 for the first 4 min. of the g.c. run, and then switched on *m/e* 154 with the aid of the peak matching unit. A typical s.i.m. trace is given in Fig. 1. Frequent checks of the atomic composition of the eluting material were made at high resolution (resolving power > 15 000). The mass spectra of the xylylcarbamoylpiperidine local anaesthetics all show a base peak due to cleavage with charge retention of the *N*-alkylated piperidine ring, and in the spectra of bupivacaine and pentyl PPX these base peaks occur at *m/e* 140 and 154 respectively (see Fig. 2). In both cases, these peaks account for 85–90% of the total ion current. With this procedure, the increased sensitivity given by single ion monitoring was applied both to bupivacaine and to the internal standard.

Analytical procedures

Assay of bupivacaine in maternal blood. Whole blood (1 ml) was placed in a 7 ml glass-stoppered tube and pentyl PPX (1 μg: 10 μl of a 100 μg ml^{-1} solution) added. It was made alkaline with 5M-NaOH (0.5 ml) and extracted for 15 min with freshly distilled diethyl ether (2×1.5 ml). The combined ethereal extracts were placed in a second 7 ml glass-stoppered tube and

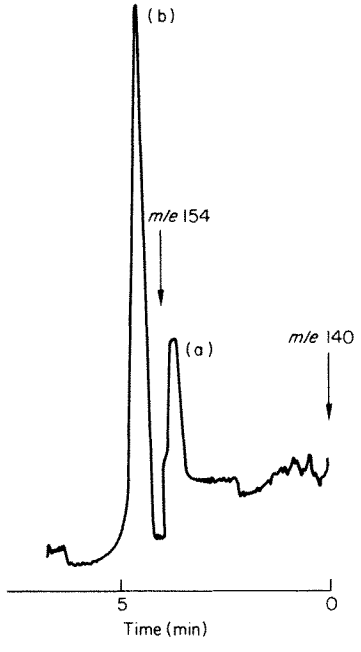

Figure 1. Chromatogram of blood extract from a fetus taken 60 min after the epidural injection of 45 mg bupivacaine to the mother. The mass spectrometer was focused on *m/e* 140 for the first 4 min of the g.c. run to record the elution of bupivacaine (a) and then switched to *m/e* 154 to record the elution of the internal standard, pentyl PPX (b). The bupivacaine concentration was 23 ng ml^{-1} and the volume of the blood sample was 73 μl. The extraction procedure and instrumental conditions are described in the text.

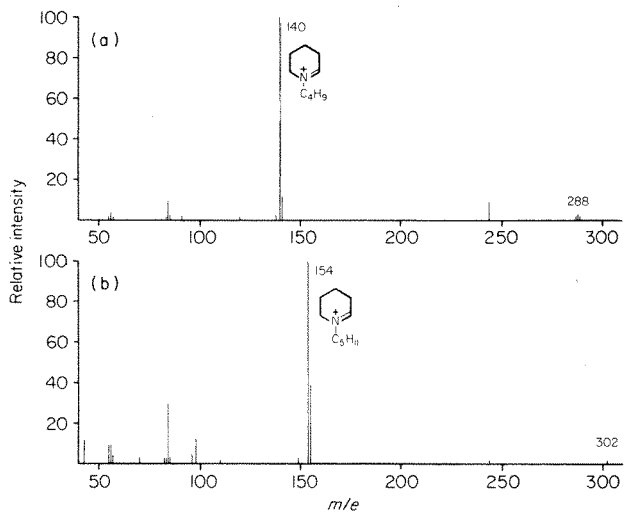

Figure 2. Electron impact mass spectra of (a) bupivacaine and (b) pentyl PPX recorded by g.c.m.s. as described in the text.

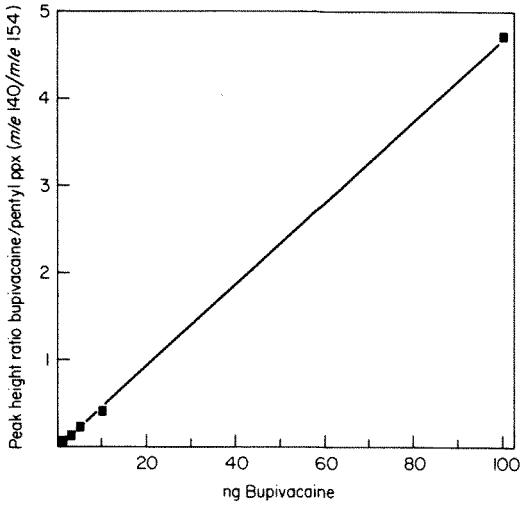

Figure 3. Calibration curve for bupivacaine recovered from 100 μl blood using pentyl PPX as the internal standard. Each point is the mean of at least three determinations ($R = 0.995$) and errors are described in the text.

extracted with M-HCl (1 ml). After centrifuging to separate the layers, the ether layer was discarded and the aqueous phase made alkaline with 5M-NaOH (0.5 ml). This was extracted with ether (2 × 1.5 ml) and the combined ethereal extracts evaporated in a 'silli-vial' (Pierce Chemical Co.) under a stream of dry N_2. The residue was dissolved in carbon disulphide (10 μl) and aliquots (1–2 μl) were taken for g.l.c.

Bupivacaine concentration in maternal blood was calculated from the peak height ratio bupivacaine/pentyl PPX, using a previously established standard curve, which was linear over the range 100–2000 ng bupivacaine. Reproducibility of assay from blood at 100 ng ml^{-1} was ± 5% (s.d., $n = 6$).

Assay of bupivacaine in fetal and neonatal blood. The small size of the fetal and neonatal blood samples (20–100 μl) did not permit the accurate measurement of their volume, and the blood volume was obtained by weighing the sample tube, adding water (1 ml) and mixing with a vortex mixer for 2 min. The diluted blood was transferred to an extraction tube, and 5M-NaOH (0.5 ml) placed in the sample tube. After vortexing for 2 min this was added to the diluted blood and the sample tube placed in a desiccator over P_2O_5 overnight. The tube was then re-weighed, and the weight of blood originally present converted to volume by the factor 1.055, the specific gravity of fetal blood.[7]

To the diluted alkaline blood was added pentyl PPX (100 ng; 10 μl of a 10 μg ml^{-1} solution), and the extraction was carried out as described above. After evaporation of the final ethereal extract, the residue was dissolved in methanol (25 μl), and aliquots (3–5 μl) injected on the column of the g.c.m.s. The g.c. effluent was subjected to s.i.m. as described, and the amount of bupivacaine present was obtained from the peak height ratio m/e 140: m/e 154, reference being made to a previously established standard curve, which was linear over the range 1–100 ng bupivacaine. This is shown in Fig. 3. At 10 ng the reproducibility of assay from blood was ± 6% (s.d., $n = 5$) while at 1 ng this rose to ± 13% (s.d., $n = 5$).

RESULTS AND DISCUSSION

The blood levels of bupivacaine in mothers and their babies after epidural injection of this drug to the mothers are shown in Fig. 4. Bupivacaine entered the maternal circulation very rapidly after epidural injection, and the peak concentration of 202 ± 38 ng ml^{-1} (mean ± s.e.) was obtained within 5 min; thereafter the levels declined monophasically. Bupivacaine passed rapidly from the maternal circulation to that of the fetus, being detectable in fetal scalp blood as early as 10 min after its administration to the mother. At 20 min post injection, the concentration of bupivacaine in fetal blood was 29 ± 6 ng ml^{-1} and the feto-maternal ratio was 0.168, while at 60 min post injection, the fetal blood

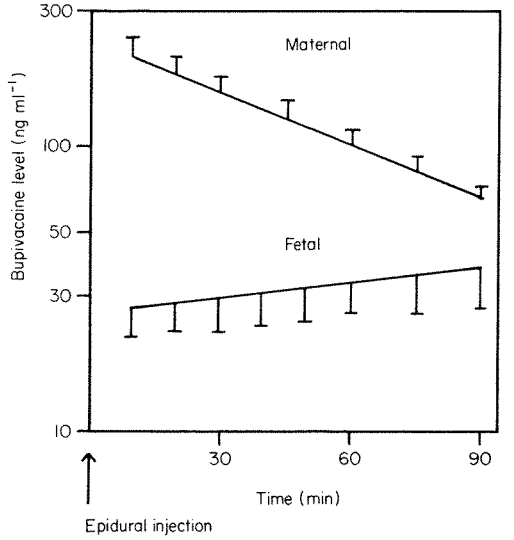

Figure 4. Log blood concentrations of bupivacaine in mothers and their fetuses after the epidural injection of bupivacaine (dose 35 ± 3.2 mg s.e.) to the mothers. Maternal blood bupivacaine was estimated by g.l.c. and fetal blood bupivacaine by g.c.m.s. as described in the text. Each point indicates the mean ±s.e. of six subjects.

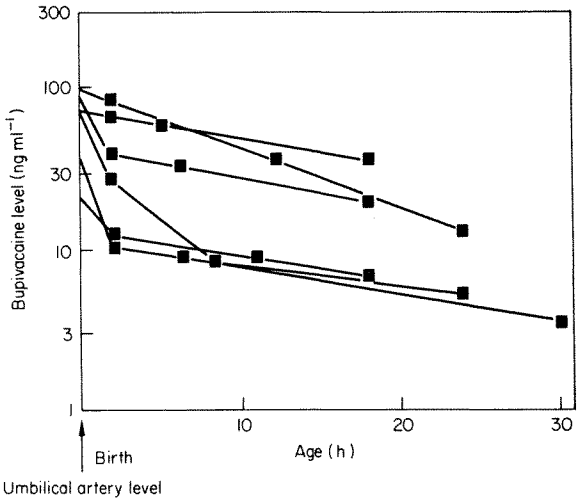

Figure 5. Log blood level/time curves of bupivacaine in six babies born of mothers who received bupivacaine by epidural injection during labour. The terminal phase of each curve was used to calculate the blood half-life of bupivacaine in the infants.

bupivacaine concentration had risen to 34 ± 8 ng ml^{-1} and the feto-maternal ratio was 0.34. At the end of the sample collection period, labour proceeded normally and further injections of bupivacaine were given as required by the mother. At delivery, the mean concentration of bupivacaine in maternal venous blood was 224 ± 32 ng ml^{-1} and in the umbilical vein and artery the values were 66 ± 11 and 56 ± 12 ng ml^{-1}, respectively. A two-tailed paired t test gave a result of $0.1 < p > 0.05$. This difference between the umbilical venous and arterial concentrations suggests that bupivacaine is transferred from the mother to her fetus and indicates some disappearance of the drug from the fetal circulation.

The sensitivity and specificity of the g.c.m.s. assay for bupivacaine made it possible to measure bupivacaine in samples of neonatal capillary blood obtained serially from 2 to 24 h after delivery. The mean bupivacaine concentration in the blood of babies 2 h old was 40 ± 12 ng ml^{-1} and this declined monoexponentially thereafter with a half-life of 18 ± 2 h. The individual blood level/time-curves for the six infants are shown in Fig. 5.

The rate of disappearance of bupivacaine from the blood of newborn babies was far slower than that found in adults. Caldwell *et al.*[8] and Scott *et al.*[9] both report a value of 1.25 h for the plasma half-life of bupivacaine in adults, and this contrasts with a half-life in neonates reported here of 18 h. Only very small amounts of bupivacaine are recovered in urine after its administration, which indicates that its disappearance from blood is due to metabolism rather than renal elimination. This suggests that the neonate is less able to metabolize this drug than is the adult, a finding not unexpected since work with amylobarbitone[10] and antipyrine[11] has shown that the oxidative metabolism of these drugs is markedly impaired in the newborn.

Acknowledgements

We thank Miss M. O. Cawston, Research Midwife, and the staff of the Labour Ward, St. Mary's Hospital, for their co-operation with this study. We are grateful to the Wellcome Trust and Duncan, Flockhart & Co. Ltd, the Cancer Research Council and the Joint Research Board, St Bartholomew's Hospital for financial support. All studies reported here had the approval of the Ethical Committee of St Mary's Hospital and Medical School.

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Received 22 November 1976