MATERNAL–FETAL DISTRIBUTION OF BUPIVACAINE IN THE RABBIT

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It has long been established that fetal:maternal plasma concentration (F:M) ratios of bupivacaine are relatively low at delivery following extradural analgesia [1]. It has been claimed that such low ratios might result from extensive tissue uptake [2], rather than slow placental transfer, even though ratios do not increase consistently with dose–delivery interval [3], as would be expected during tissue equilibration. Rabbit placental perfusion experiments have demonstrated consistently slow transfer of bupivacaine, associated with high maternal protein binding [4–6], but these experiments cannot measure tissue uptake or equilibration. Progressive accumulation of bupivacaine in brain and other tissues on prolonged maternal administration might result in serious detriment to the offspring. In order to establish if such accumulation does occur, we have examined in rabbits the uptake of bupivacaine in feto–placental units removed serially during maternal steady-state infusion. A brief report of part of this work has appeared elsewhere [7].

SUMMARY

Bupivacaine was infused i.v. in nine anaesthetized pregnant rabbit does near term. Pups were removed at 10–15 min intervals and bupivacaine concentrations measured in fetal plasma, brain, placenta, amniotic fluid, maternal plasma sampled synchronously and maternal brain at the end of the experiment. Mean maximum fetal:maternal (F:M) ratio was 0.31 (SD 0.16) (range 0.18–0.64). Mean fetal brain:plasma ratios ranged from 2.04 to 5.09. There was no progressive increase in fetal brain bupivacaine concentration with time. Maternal brain:plasma ratio was 1.62 (0.81). However, maximum fetal brain concentration was only 0.27–0.86 of maternal. Concentrations increased with time in amniotic fluid, but did not exceed those in maternal plasma. Although there was some accumulation of bupivacaine in rabbit fetuses, tissue uptake could not account for low F:M ratios persisting beyond 80 min.

MATERIALS AND METHODS

The experiments were conducted on nine pregnant New Zealand white rabbits (weights 3.8–4.9 kg (mean 4.4 (SD 0.4) kg) within 4 days of term (gestation = 30 days). The does were anaesthetized using alphaxalone/alphadolone and urethane as described previously [6] and they breathed air spontaneously through a tracheostomy. A carotid artery was cannulated for monitoring of arterial pressure and sampling. Bupivacaine 1.25 mg ml⁻¹ was infused into a neck vein at a rate of 12 ml h⁻¹ for 20 min as a loading dose, followed by 6 ml h⁻¹ for 1 h, then 3 ml h⁻¹ thereafter. Single fetal sacs were removed through a series of hysterotomies at intervals of approximately 15 min, sampling maternal plasma synchronously on each occasion. The first fetal sac was removed 6–20 min after the start of the maternal infusion of bupivacaine, except in two does, when it was delayed for 72 and 77 min to allow time for fetal equilibration in the absence of maternal insults. Amniotic fluid was collected, each fetus decapitated and blood collected via a funnel into a heparinized tube; the placenta and the brain were removed and weighed. The maternal brain was removed likewise at the end of the experiment.

All tissues were homogenized in hydrochloric acid 0.1 mol litre⁻¹ using an Ultraturax homogenizer. Homogenate volumes were between 7.5
and 20 ml, and 2.5-ml samples were assayed. Bupivacaine concentrations were measured in all samples of tissue, amniotic fluid (0.5 ml) and plasma (0.1-0.5 ml) by gas-liquid chromatography following a three-stage extraction process, and using a nitrogen detector, with 1-ethyl-2', 6'-pipercoxylylidine as internal standard. Maternal arterial pH and blood-gases were analysed before and after the removal of all fetal sacs. Results are expressed where appropriate as mean (SD). Student's t tests (paired and unpaired) were used to test for significant differences between means. Reciprocals of concentration and time were calculated in order to determine the time to 50% equilibration into amniotic fluid.

**RESULTS**

There was a significant reduction in mean arterial pressure, but not pH or blood-gas values, between the beginning and end of the experiment (table I). Does had between four and 11 fetuses (mean 8). The weights of the does, pups, placentae and brains are shown in table II.
The intrapair correlation for duplicate bupivacaine analyses was > 0.99 (n = 35). Coefficient of variation for the assay was 5%.

Figure 1 shows typical data, from a single doe and her fetuses, and data from one of the does in which sampling was delayed. Bupivacaine concentration was consistently higher in maternal plasma than that in any fetal compartment.

Individual F:M ratios (fig. 2) all increased for the first 30-40 min and thereafter there was no consistent trend. The mean (SD) maximum F:M ratio was 0.31 (0.16) and the range 0.18–0.64.

Fetal brain concentration (fig. 3) also increased initially in five of the seven animals sampled early, again with no consistent changes thereafter. Maximum fetal brain bupivacaine concentration in a given litter ranged from 27 to 86% of the doe's brain concentration. The highest fetal brain concentration occurred at 58–178 min from the start of the infusion (mean 101 (39)). Fetal brain:plasma ratio ranged from 1.1 to 6.5 (means 2.0–5.1) and did not increase with time but demonstrated, if anything, a downward trend (fig. 4). The mean maximum fetal brain:plasma ratio was 4.8 (1.3) and was significantly greater (P < 0.001) than the mean maternal brain:plasma ratio of 1.6 (0.8) (range 0.68–3.1).

Placental bupivacaine concentration increased in five does for between 30 and 80 min, but otherwise showed no marked changes (fig. 5). Only bupivacaine concentrations in amniotic fluid showed a clear upward trend, and appeared to plateau at approximately 90 min (fig. 6) (time to half maximum = 27.6 min).

**DISCUSSION**

Rabbits were selected as the experimental model because progressive fetal tissue accumulation could be measured by making use of the large
litters. This advantage was thought to outweigh the inconvenience of small fetal plasma volumes.

A degree of accumulation was noted in the rabbit fetal compartment, but it was not sufficient to explain observed low F:M plasma ratios which, although sometimes erratic, mostly remained less than 0.3. The occasional high values shown in figure 2 may be explained by the presence of acidosis in some pups, which would promote ion trapping in fetal plasma. Kennedy and col-

![Graph showing fetal brain:plasma ratios (n = 9).]

**Fig. 4.** Fetal brain:plasma ratios (*n* = 9).

![Graph showing placental concentrations of bupivacaine (logarithmic scale) in all does.]

**Fig. 5.** Placental concentrations of bupivacaine (logarithmic scale) in all does.

![Graph showing amniotic fluid concentrations of bupivacaine (logarithmic scale) in all does.]

**Fig. 6.** Amniotic fluid concentrations of bupivacaine (logarithmic scale) in all does.
leagues [8] demonstrated a significant increase in F:M bupivacaine ratio in the presence of an increased transplacental pH gradient, in sheep. While the does themselves were not acidotic in the present study, and their circulations not greatly impaired by anaesthesia, repeated hysterotomy and removal of conceptus (table I), it was impossible to ensure that all fetuses were not acidotic. Indeed, the variation in size among litter mates attests to the variability in function among the placentae. It was not possible to measure fetal plasma pH because all the small volume of fetal blood available was required for analysis of bupivacaine, and because the method of collection precluded it. It was to eliminate the possible effect of repeated maternal insults on long term distribution of bupivacaine that removal of conceptuses was delayed in two experiments, when bupivacaine concentrations were observed to be reasonably stable in all compartments (figures 1B, 3 and 5).

Using the sheep model, Kennedy and his associates [9] found that mean F:M bupivacaine ratios increased progressively to approximately 0.3 after 1 h of maternal infusion with no significant difference at that time between umbilical venous (UV) and fetal arterial (FA) concentrations, suggesting little continuing uptake. After infusion was stopped, F:M ratios increased further and FA exceeded UV, denoting back transfer despite F:M ratios of 0.3-0.5. It is now well established in humans that low F:M bupivacaine ratios at delivery following extradural administration are associated with reduced protein binding in the fetus, with negligible gradient for free drug [10, 11]. The concentration of the principal binding protein for bupivacaine, α1-acid glycoprotein, is lower in fetus than mother, even at term, in both man [12] and rabbit [5].

Fetal brain concentration of bupivacaine exhibited no consistent upward trend, and indeed fetal brain:plasma ratio appeared to decline with time. This may be explained by the superior buffering power of the brain in the face of systemic acidosis. A study in non-acidotic, non-pregnant mice [13] has confirmed a constant relationship between blood and brain concentrations of bupivacaine at 2-40 min following bolus i.v. injection. Indeed, in mice, there was no evidence of significant tissue uptake of bupivacaine beyond 2 min into any of the vessel-rich group.

Of the compartments examined in the present study, the only one showing any evidence of slow accumulation of bupivacaine was amniotic fluid. Drugs which undergo rapid renal excretion appear quickly in amniotic fluid following placental transfer [14]. For a lipid soluble drug such as bupivacaine (partition coefficient in oleyl alcohol/pH 7.4 buffer = 257 [4]), in which renal excretion of unchanged drug plays only a small part in elimination [15], the amniotic fluid represents a very slow compartment, which probably equilibrates directly with the maternal compartment across the amnion [14].

Being lipid soluble, bupivacaine accumulates in fat, but more slowly than in the vessel-rich group of tissues. The fetus, however contains too little fat from which to extract bupivacaine.

In the present study there was no trace of piperoclyxylidine, the initial metabolite of bupivacaine, in any fetal tissues. It does not seem likely, therefore, that metabolism of bupivacaine could contribute to the low concentrations found in the fetus.

Low fetal plasma binding may account for the fact that brain:plasma ratios of bupivacaine were higher in the fetus than in the mother. Nevertheless, fetal brain concentrations rarely exceeded maternal plasma or brain concentrations. Brain concentrations are a determinant of bupivacaine toxicity and it is clear that, in rabbits, bupivacaine does not accumulate in fetal brain during continuous maternal administration. These findings do not support the concept that there might be clinically important accumulation of bupivacaine in fetal tissues as a result of prolonged administration of the drug to the mother.

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REFERENCES

DISTRIBUTION OF BUPIVACAINE