The extradural administration of fentanyl has been found to relieve the pain of labour (Justins et al., 1982) when the associated plasma concentration may exceed 2 ng ml⁻¹ (Justins et al., 1983). Usually, fentanyl is given with bupivacaine, either successively or concurrently, to a mother who may also have received pethidine before choosing extradural analgesia (Vella et al., 1985).

We have, therefore, studied the transfer of fentanyl, bupivacaine and pethidine across the rabbit placenta perfused in situ using a modification of the method first described by Money and Dancis (1960), and have examined the effect of the concurrent administration of the latter two drugs on the transfer of fentanyl.

MATERIALS AND METHODS

Seven pregnant New Zealand White rabbits weighing between 3.6 and 5.0 kg and at 26–29 days gestation were studied. Anaesthesia was induced with Althesin 0.5 ml and maintained with 25% ethyl carbamate (urethane) to a maximum of 1.5 g kg⁻¹ via a 23-gauge "Butterfly" in an ear vein. The doe's temperature was maintained using a homeothermic blanket (Ealing Berk Ltd) set to 39 °C.

A tracheal cannula was inserted, through which the doe breathed room air spontaneously. A carotid artery was cannulated to permit the continuous monitoring of arterial pressure, and the sampling of blood for drug and gas analyses. Isotonic solutions of the relevant drugs were infused via a neck vein.

SUMMARY

The transfer of fentanyl has been studied in the perfused in situ rabbit placenta. Does were infused with fentanyl and, subsequently, with fentanyl plus bupivacaine and pethidine. Antipyrine was infused throughout as an index of materno–fetal exchange. The fetal side of one placenta was perfused artificially with Krebs buffer containing dextran, and the effluent collected from the umbilical vein (uv). Drug concentrations were measured in maternal arterial blood (ma) and uv. Maternal plasma protein binding was also measured. Transfer of fentanyl was intermediate between that of pethidine and bupivacaine ($C_{uv}:C_{ma}$ for fentanyl was 0.25, for bupivacaine, 0.11 and for pethidine was 0.44), while placental clearance ($C_{uv} \times$ umbilical flow/$C_{ma}$) increased with umbilical flow between 0.5 and 4.0 ml min⁻¹ for all drugs. When clearances of unbound drugs were calculated, those of bupivacaine, pethidine and antipyrine were similar, but that of fentanyl was higher. The clearance of fentanyl was not affected by the presence of bupivacaine and pethidine.

During phase I, fentanyl 2 μg ml⁻¹ was infused, and during phase II, fentanyl 2 μg ml⁻¹, bupivacaine 2 mg ml⁻¹ and pethidine 2 mg ml⁻¹. Antipyrine 4 mg ml⁻¹ was infused throughout, as an index of placental exchange (Meschia et al., 1967). During each phase, the infusion rate was 6 ml h⁻¹ for the first 30 min and 3 ml h⁻¹ thereafter.

Placental preparation

The uterus was approached via a median abdominal incision, a single fetal sac was opened, the umbilical arteries and umbilical vein were...
cannulated with 22-gauge and 20-gauge cannulae (Medicut), respectively, and the fetus was removed. The uterine segment was kept warm (38°C ± 1°C) and moist by covering it with a sheet of thin plastic under an unfocused lamp. The fetal side of the placental circulation was perfused via the two umbilical arteries using a peristaltic pump (Harvard) with a Kreb's bicarbonate buffer (pH 7.40 ± 0.06) gassed at 38°C with 5% carbon dioxide in oxygen and containing (mmol litre⁻¹): NaCl 118.40, KH₂PO₄ 1.20, KCl 4.75, MgCl₂ 128, NaHCO₃ 25.00, CaCl₂ 2.55 and MgSO₄ 1.23, to which 3% Dextran 40 was added. A non-recirculating system was used.

**Procedure**

Umbilical flow rate was varied in two-fold steps between 0.5 and 4.0 ml min⁻¹ during each phase. The effluent umbilical perfusate of the first 10 min at each rate was discarded, and two 5-ml collections were made thereafter from the umbilical vein. A maternal arterial blood sample was taken at the midpoint of the collections, and replaced with an equal volume of 0.9% sodium chloride solution. Maternal blood-gas tensions were measured at the midpoint of each study, and at the end 20 ml of maternal blood was taken for the estimation of protein binding by ultrafiltration (Reynolds et al., 1976). All blood samples were heparinized and the plasma was separated for drug analysis. Partition coefficients were obtained by shaking oleyl alcohol 0.5 ml with Sorensen's buffer 9.5 ml (pH 7.4) containing fentanyl, bupivacaine and pethidine.

**Drug analyses**

In maternal plasma, effluent umbilical perfusate and plasma ultrafiltrate, antipyrine, bupivacaine and pethidine were analysed by gas chromatography as described elsewhere (Hamshaw-Thomas, Rogerson and Reynolds, 1984). Fentanyl concentrations were measured by radio-immunoassay (Michels, Hendriks and Heykants, 1977).

**Statistical analyses**

An intrapair correlation between successive perfusate drug concentrations was used to test the constancy of placental transfer. Paired t tests were used to examine the effect of umbilical flow rate on fetal-maternal ratios of each drug, and of concurrent drug administration.

**RESULTS**

Table I shows the maternal blood-gas tensions and mean arterial pressure during both phases. Maternal blood-gas tensions were in agreement with those previously recorded for rabbits (Kozma et al., 1974). The difference in arterial pressure between the two phases of the experiment was not significant.

<table>
<thead>
<tr>
<th>Table I. Maternal physiological data</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
</tr>
<tr>
<td>PCO₂ (kPa)</td>
</tr>
<tr>
<td>PO₂ (kPa)</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
</tr>
<tr>
<td>Phase I</td>
</tr>
<tr>
<td>Phase II</td>
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</tbody>
</table>
Figure 1 shows the absolute concentrations of the individual drugs in maternal plasma and umbilical perfusate of one preparation. The intrapair correlations for drug concentrations in successive umbilical perfusates in all rabbits are given in table II. Table III shows the mean maternal clearance of the drugs. There was considerable inter-rabbit variation in antipyrine clearance, but no significant difference between phases I and II. The clearance of fentanyl decreased, presumably as a result of decreases in the tissue uptake of this drug with its large distribution volume.

Table IV shows the mean values for the

<table>
<thead>
<tr>
<th>Flow rate (ml min⁻¹)</th>
<th>Fentanyl</th>
<th>Antipyrine</th>
<th>Pethidine</th>
<th>Bupivacaine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I (n = 6 throughout)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0.273 ± 0.170</td>
<td>0.653 ± 0.253</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0.262 ± 0.172</td>
<td>0.845 ± 0.170</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>0.284 ± 0.223</td>
<td>0.626 ± 0.281</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>0.297 ± 0.173</td>
<td>0.454 ± 0.284</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0.273 ± 0.170</td>
<td>0.700 ± 0.362</td>
<td>0.477 ± 0.146</td>
<td>0.106 ± 0.028</td>
</tr>
<tr>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td>(n = 4)</td>
<td>(n = 3)</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0.212 ± 0.113</td>
<td>0.590 ± 0.235</td>
<td>0.413 ± 0.141</td>
<td>0.101 ± 0.012</td>
</tr>
<tr>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>0.202 ± 0.141</td>
<td>0.510 ± 0.161</td>
<td>0.454 ± 0.032</td>
<td>0.125 ± 0.013</td>
</tr>
<tr>
<td>(n = 5)</td>
<td>(n = 3)</td>
<td>(n = 4)</td>
<td>(n = 4)</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>0.288 ± 0.203</td>
<td>0.453 ± 0.275</td>
<td>0.354 ± 0.090</td>
<td>0.110 ± 0.088</td>
</tr>
<tr>
<td>(n = 3)</td>
<td>(n = 3)</td>
<td>(n = 3)</td>
<td>(n = 4)</td>
<td></td>
</tr>
</tbody>
</table>
umbilical venous: maternal arterial concentration ratios (C_{uv}:C_{ma}) at each flow rate. Experiments were excluded if the antipyrine ratio at 1 ml min^{-1} was less than 0.5, as ratios below this value suggest a decline in placental exchange (Young, 1980). There was no significant change in antipyrine ratios between phases I and II, indicating the continued integrity of the materno-fetal exchange area. The C_{uv}:C_{ma} values for fentanyl were similar at all flow rates and there was no significant change with concurrent administration of bupivacaine and pethidine.

The maternal placental clearance of the drugs has been calculated from the equation: C_{uv} \times \text{umbilical flow rate}/C_{ma}. Figure 2 shows placental clearances of fentanyl and antipyrine during phases I and II. There were no significant differences between the placental clearance rates at individual flow rates in the two phases.

Figure 3 depicts the placental clearances of all four drugs during phase II. The clearance increased approximately linearly with increasing flow rate and was highest for antipyrine and lowest for bupivacaine.

Table V shows the molecular weights, plasma protein binding and partition coefficients of the four drugs.

Using the mean maternal protein binding data, it was possible to estimate the clearance of the unbound fraction of each drug during phase II (fig. 4). The clearances of unbound pethidine, bupivacaine and antipyrine were similar but the clearance of unbound fentanyl was significantly greater than the others at 4 ml min^{-1} (P < 0.02).
DISCUSSION

Although the rabbit placenta is haemodichorial whereas the human type is haemomonochorial, (Enders, 1965), Thornburg and Faber (1977) found that, at term, the barrier thickness was similar in the two species. However, the patterns of placental flow differ. The labyrinthine rabbit placenta possesses a countercurrent flow pattern (Mossmann, 1926; Carter, 1975): that is, blood in the fetal capillary net flows from the endometrial towards the chorionic surface, whereas maternal blood flows in the opposite direction. In contrast, the multivillous human placenta appears to function as a less efficient cross-current (Moll, 1972) or even a concurrent system. In the present non-recirculating experiments, a counter-current system produces a better estimate of transfer in life, since umbilical perfusate tends to equilibrate with maternal arterial rather than uterine venous blood, thus a greater degree of equilibration is possible after a single circulation than would be observed in a concurrent flow system.

Gas chromatography permits the assay of individual drugs that have been given simultaneously. This allows a direct comparison to be made between drugs, while eliminating inter-rabbit variation. Fentanyl concentrations were too low to interfere with the assay. The wide variation in maternal fentanyl clearance is in agreement with other studies in rabbits (Rigg et al., 1981).

Placental clearance, that is the volume of maternal blood cleared of drug by the placenta in unit time, may be calculated from the formula:

\[
\frac{(C_{uv} - C_{ub}) \times Q_u}{C_{ma}}
\]

where \(Q_u\) = effective umbilical blood flow. \(C_{ub}\) is zero in the present study. Using clearance as a measure of placental transfer ignores placental uptake of drug, which would be significant only during the early part of any experiment and can therefore be neglected, as is confirmed by the significant correlation between drug concentrations in paired perfusate collections (table II).

All the drugs show flow-dependent transfer, that is clearance increases with umbilical flow, suggesting that at low flows equilibration occurs, but maternal blood is not cleared completely of drug. This is in agreement with data previously published (Hamshaw-Thomas, Rogerson and Reynolds, 1984).

The placental transfer of fentanyl is intermediate between bupivacaine and pethidine. Although fentanyl and bupivacaine are equally bound to plasma proteins in our rabbits, \(C_{uv}:C_{ma}\) for fentanyl is higher than for bupivacaine. This is unlikely to be explained by the slightly higher lipid solubility of fentanyl, since the much greater differences in lipid solubility between the other three drugs have no such effect (Hamshaw-Thomas, Rogerson and Reynolds, 1984). Since a compound with the lipid solubility of pethidine exhibits flow-dependent transfer, any further increase in lipid solubility is unlikely to enhance transfer in the conditions of the present study.

In these investigations the fetal blood supply to the placenta was replaced with a protein-free solution containing low molecular weight dextran which was added to maintain oncotic pressure and reduce placental oedema, and which does not bind drugs (Hamshaw-Thomas and Reynolds, 1985). Thus only unbound drug can be expected to equilibrate across the placenta. The fetal:maternal ratios for bupivacaine and pethidine are, therefore, lower than previously recorded for human pregnancies (Moore, McNabb and Glynn, 1973; Thomas et al., 1976). For fentanyl, human fetal:maternal ratios measured in this laboratory have been around 0.8 (unpublished data). We have found no published data for this drug. Carrie and O’Sullivan (1981) measured maternal and fetal concentrations of fentanyl after extradural injection, but ratios could not be obtained because samples were not taken simultaneously.

Fentanyl is bound to serum albumin (Bower, 1981), whereas bupivacaine and pethidine are bound principally to \(\alpha_1\)-acid glycoprotein (Piafsky and Knoppert, 1978; Holmberg et al., 1982). During gestation the serum albumin concentration in the fetus increases to exceed the decreasing maternal albumin concentration at term whereas fetal \(\alpha_1\)-acid glycoprotein never exceeds the maternal concentration (Krauer, Dayer and Anner, 1984). Thus equilibrium fetal:maternal ratios will be higher for fentanyl than for bupivacaine. Studies in pregnant ewes (Croft et al., 1981) have shown low fetal:maternal ratios of fentanyl (0.33) after single bolus injections, but the adequacy of placental exchange was not validated by simultaneous antipyrine ratios.

Transfer of fentanyl across the placenta was unaffected by the other drugs, presumably because of their different binding characteristics. When the transfer of unbound drugs was calculated, the placental clearances of bupivacaine,
pethidine and antipyrine were indistinguishable. Fentanyl clearance was, however, higher and there was no significant difference between umbilical venous and maternal free fentanyl concentrations. In both phases I and II, the clearance of unbound fentanyl of > 4 ml min⁻¹ at the highest umbilical flow rate was significantly higher than that of the other drugs, and is likely to have exceeded the maternal flow rate (Duncan and Lewis, 1969). Drug binding to albumin, although of larger capacity, generally shows lower affinity than that to \( \alpha_1 \)-acid glycoprotein (Coyle et al., 1984). It would appear, therefore, that fentanyl, unlike bupivacaine (Hamshaw-Thomas and Reynolds, 1985) can unbind from albumin in a single transit of the placenta, thus free fentanyl crossing the membrane is replaced from the maternal store of bound fentanyl.

In this study, a pH gradient as a source of variability in fetal:maternal ratios of weak acids and bases (Reynolds, 1983), has been eliminated.

**CONCLUSION**

Although fentanyl, like bupivacaine and pethidine, shows flow dependent clearance, it crosses the placenta more readily than bupivacaine. Its transfer is not affected by the presence of the other two drugs.

**REFERENCES**


