RELATIONSHIP BETWEEN THE TRANSPLACENTAL GRADIENTS OF BUPIVACAINE AND α_1 -ACID GLYCOPROTEIN

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- 1 The binding of bupivacaine (400 ng/ml) to isolated α_1 -acid glycoprotein was studied at two protein concentrations. At 20 mg/100 ml the extent of bupivacaine binding was $31.0 \pm 1.8\%$ (mean $\pm$ s.d., $n = 4$), and at a protein concentration of 60 mg/100 ml binding of bupivacaine was $85.8 \pm 1.5\%$ ($n = 4$).
- 2 Bupivacaine and α_1 -acid glycoprotein concentrations were measured in plasma samples collected from a maternal peripheral vein and the umbilical vein at delivery ($n = 23$). The ratio of the foetal:maternal bupivacaine concentrations ranged from 0.17 to 0.52, while the foetal:maternal ratio for α_1 -acid glycoprotein concentrations ranged from 0.20 to 0.96. A positive relationship emerged between the two ratios ($P < 0.01$).
- 3 The α_1 -acid glycoprotein concentration gradient across the placenta, and interindividual variability in the gradient appear to contribute to the low and variable transplacental bupivacaine concentration ratio observed.

Introduction

Bupivacaine is commonly used as an epidural analgesic in obstetrics to provide relief from pain during labour and delivery. The transplacental plasma concentration gradient of bupivacaine has been reported by several groups (Thomas, Climie & Mather, 1969; Geerinckx *et al.*, 1974; Tucker *et al.*, 1970) and while it was found that the plasma concentration was always higher in the mother (BUP_M) than in the foetus (BUP_F), marked interindividual variability in the ratio (BUP_F/BUP_M) was noted by all workers. The protein binding of bupivacaine in maternal plasma and in plasma from the umbilical vein has been studied to determine whether differences in plasma binding across the placenta could account for the generally low BUP_F/BUP_M ratio (Tucker *et al.*, 1970; Mather, Long & Thomas, 1971) and the intersubject variability observed (Thomas *et al.*, 1976). Tucker *et al.* (1970) found that at a concentration of 1 μ g/ml, 95% was bound in maternal plasma while only 66% was bound in foetal plasma and that the plasma binding of bupivacaine was concentration dependent.

Mather *et al.* (1971) confirmed this binding differential and also reported that only 33% of bupivacaine (0.5 μ g/ml) in a solution of human serum albumin (4.8 g/100 ml) was bound to the protein. This work suggested that differential protein binding across the placenta could play a significant part in determining the transplacental plasma concentration gradient of bupivacaine, and also that proteins other than albumin must be involved in its plasma binding. Further evidence was gained from the work of Thomas *et al.* (1976) who found that the concentration of unbound bupivacaine was not significantly different on either side of the placenta, and that intersubject variation in the BUP_F/BUP_M ratio was due largely to individual variation in the relative extents of protein binding of bupivacaine in maternal and foetal plasma. A significant correlation was found between BUP_F/BUP_M and the ratio of total protein concentration in umbilical and maternal plasma.

Recently, several basic drugs including bupiva-

caine have been shown to bind to the acute-phase protein α_1 -acid glycoprotein (AAG) (Piafsky, 1980). AAG has a marked concentration differential across the placenta, with plasma levels in the foetus only about one third of those in the mother (Laurell, 1968). It is possible, therefore, that this protein may be partially responsible for the differential plasma binding of bupivacaine across the placenta, and hence the resultant low and variable concentration ratio BUP_F/BUP_M . Thus study was undertaken to examine the role AAG plays in the transplacental distribution of bupivacaine.

Methods

Subjects and blood collection

Twenty-two women who required epidural anaesthesia with bupivacaine prior to delivery were included in this study. Seventeen patients delivered vaginally after oxytocin-induced or spontaneous labour and five delivered by caesarean section (three emergency and two elective); there was one set of twins. The duration of labour varied from 2.0 to 26.5 h; the duration of second stage ranged from 0.2 to 3.1 h. In the hours prior to delivery some patients received diazepam (5 patients), nitrazepam (4), promazine (2), pethidine (1), chloral hydrate (1), hyoscine (1) and pentobarbitone (1); four patients delivered under a general anaesthetic consisting of thiopentone, atropine, suxamethonium and nitrous oxide.

Blood samples were collected at delivery by venepuncture from an antecubital vein of the mother (10 ml) and from the umbilical vein (10–20 ml) of the clamped cord after delivery of the placenta. Samples were placed in plastic tubes (10 ml) containing ammonium heparin (125 IU) and sedimentation granules (Disposable Products, Sydney, Australia). After centrifugation the plasma was frozen at -22°C prior to analysis for bupivacaine and AAG.

Measurement of bupivacaine and AAG concentrations

Plasma samples were analysed for bupivacaine using the gas chromatographic method of Mather *et al.* (1971). The only major modification to this method was the use of mepivacaine as internal standard. Radial immunodiffusion plates (M-Partigen; Behringwerke AG, Marburg, W. Germany) were used to measure the AAG concentrations in plasma from the umbilical and maternal vein. The coefficients of variation of the bupivacaine and AAG assays at clinically relevant concentrations were 4.7% ($n = 5$) and 2.9% ($n = 6$), respectively.

Bupivacaine protein binding estimations

The binding of bupivacaine to isolated AAG (Behringwerke) was determined using an equilibrium dialysis method. Perspex cells with a capacity of 0.75 ml were separated by Visking dialysis membrane (molecular weight cut-off, 6,000–8,000; Visking Co., Chicago, Ill., U.S.A.) which had been washed and dried. Solutions of AAG in isotonic phosphate buffer (pH 7.4; NaH_2PO_4 , 0.013 M; Na_2HPO_4 , 0.054 M; NaCl, 0.44%) were spiked with bupivacaine (400 ng/ml) and dialysed against protein-free buffer solution. Two concentrations of AAG were studied, 20 mg/100 ml and 60 mg/100 ml, and four replicates were run at each AAG concentration. The cells were agitated gently at 37°C for 6 h at which time equilibration across the membrane had been established. Aliquots from each side of the membrane were removed and stored frozen prior to analysis for bupivacaine.

The binding of bupivacaine was also studied in umbilical venous plasma. The sample of plasma was split into two, and one part was supplemented with AAG to a concentration similar to that found in maternal plasma. AAG was measured in both plasma samples, which were then spiked with bupivacaine to a concentration of 400 ng/ml and dialysed against isotonic phosphate buffer, as described above, to determine the degree of binding.

Statistical methods

The Spearman's rank-correlation coefficient (r') was used as a quantitative index of association between variables (Freund, 1973).

Results and Discussion

In studying the binding of bupivacaine to isolated AAG, concentrations of the protein were chosen to approximate those found in umbilical venous plasma (20 mg/100 ml) and in maternal plasma (60 mg/100 ml). At an AAG concentration of 20 mg/100 ml, $31.0 \pm 1.8\%$ (mean $\pm$ s.d., $n = 4$) of the bupivacaine was bound, and the concentration of the drug on the protein side of the membrane at the completion of dialysis was 238 ± 9 ng/ml. At the higher AAG level the final bupivacaine concentration in the protein compartment was 370 ± 115 ng/ml ($n = 4$) and the extent of binding was $85.8 \pm 1.5\%$. These data complement those of Piafsky & Knoppert (1978) who found that 73% of bupivacaine was bound to isolated AAG (40 mg/100 ml) when the drug concentration was 1 $\mu\text{g/ml}$. The binding of bupivacaine in plasma collected from the umbilical vein (AAG 30 mg/100 ml) was $89.2 \pm 1.7\%$ ($n = 6$), and after augmentation to an AAG concentration of 57 mg/100 ml binding

increased to 94.4% (replicates 93.9, 94.8). Collectively these results indicate that AAG binds bupivacaine, but apparently has a low capacity for the drug, and that the AAG concentration differential across the placenta contributes to the observed differential plasma binding (Tucker *et al.*, 1970; Mather *et al.*, 1971). Also, variability in the AAG differential may account for intersubject variability in the foetal: maternal ratio of plasma bupivacaine.

In the 23 deliveries in which the transplacental plasma concentration gradient of bupivacaine was studied, the mean (0.30 ± 0.10) and range (0.17–0.52) of BUP_F/BUP_M were similar to those reported previously (Thomas *et al.*, 1969; Geerinckx *et al.*, 1974; Tucker *et al.*, 1970). Also in keeping with earlier reports (Geerinckx *et al.*, 1974; Thomas *et al.*, 1976) the ratio was not dependent on the time that elapsed between the last dose of bupivacaine and delivery ($P > 0.05$). This time difference ranged from 15 min to 185 min in this study. Plasma AAG levels were markedly different ($P < 0.01$) between the two circulations. Maternal levels (AAG_M) ranged from 39–98 mg/100 ml (57 ± 15 mg/100 ml) and AAG in umbilical venous plasma (AAG_F) ranged from 9–41 mg/100 ml (24 ± 10 mg/100 ml). The ratio AAG_F/AAG_M had values from 0.20 to 0.96, and neither AAG_M , AAG_F nor the ratio AAG_F/AAG_M was dependent on the total duration of labour or the

duration of second stage of labour ($P > 0.05$) and did not appear to depend on the mode of delivery. The values obtained for AAG_F and AAG_M were in close agreement with the data of Laurell (1968) who used an electrophoretic technique, and the data of Gleichmann *et al.* (1973) and Song *et al.* (1970) who used radial immunodiffusion methods for the measurement of AAG concentrations. The lack of correlation between maternal or foetal seromucoid (of which AAG is the major component) and the duration or mode of delivery has been reported previously (Good *et al.*, 1973). The absence of correlation is to be expected since the response of AAG to stress or trauma only becomes apparent 24–48 h after the onset of the stressful situation (Fremstad *et al.*, 1976; Ridd *et al.*, 1979), and in this study the duration of labour exceeded 24 h in only one case.

Both the BUP_F/BUP_M and AAG_F/AAG_M ratios displayed great interindividual variability (0.17–0.52 and 0.20–0.96, respectively). A positive relationship did emerge between BUP_F/BUP_M and AAG_F/AAG_M ($r' = 0.63$, $P < 0.01$, $n = 23$) (Figure 1). This indicates that the AAG ratio is partially responsible for the interindividual variability commonly observed in BUP_F/BUP_M . It is recognised, however, that other factors are likely to influence the BUP_F/BUP_M ratio. In this study, such factors appear to have decreased the strength of the correlation between BUP_F/BUP_M

Figure 1 The relationship between the ratio of bupivacaine concentrations in umbilical venous and maternal venous plasma (BUP_F/BUP_M) and the corresponding ratio of α_1 -acid glycoprotein levels (AAG_F/AAG_M) at delivery ($r' = 0.63$, $P < 0.01$, $n = 23$). ● foetal distress, ■ nitrazepam.

and AAG_F/AAG_M . There was one case of foetal distress, a condition commonly accompanied by foetal acidosis. Bupivacaine is a weak base ($pK_a = 8.1$) and it has been shown that foetal acidosis can result in ion-trapping of weak bases in the foetal circulation, resulting in an increase in BUP_F/BUP_M (Nation, 1980). It is interesting to note that this patient had a high transplacental plasma concentration gradient of bupivacaine, though AAG_F/AAG_M was towards the low end of the observed range (Figure 1). When this patient's data were excluded, r' increased to 0.68 ($P < 0.01$, $n = 22$). Four patients had each received nitrazepam (10 mg) the evening before the day of delivery; their BUP_F/BUP_M ratios were below those expected for their AAG ratios (Figure 1). Nitrazepam, like bupivacaine, is a basic drug and has two pK_a s at 3.2 and 10.8, and therefore it is expected to carry a positive charge at physiological pH. These properties are similar to those of the many drugs that have now been shown to bind to AAG (Piafsky, 1980) and so nitrazepam may also fall into that group. It is possible, therefore, that nitrazepam was causing differential displacement of bupivacaine from AAG, the effect being greater on the foetal side of the placenta where the AAG concentration was lower. When these four patients were also excluded

from the data treatment, r' rose even further to 0.84 ($P < 0.01$, $n = 18$). No other drugs appeared to have an effect on the transplacental gradient of bupivacaine.

It is most important that without excluding any data there was a significant correlation between the bupivacaine concentration ratio and the α_1 -acid glycoprotein concentration ratio across the placenta (Figure 1). This is consistent with the fact that bupivacaine was found to bind to AAG and that the degree of binding was less at the lower AAG concentration present in the foetal circulation. Therefore, the present results strongly indicate that the relative levels of this acute-phase protein on either side of the placenta are an important determinant of the bupivacaine concentration gradient. A correlation between the extent of plasma binding and AAG concentrations in maternal and foetal plasma has also been reported for lignocaine and propranolol (Wood & Wood, 1981) and for pethidine (Nation, 1981). Additional factors which may affect the BUP_F/BUP_M ratio, including the influence of other proteins (Mather & Thomas, 1978) and of pH, also need to be considered and are under investigation in this laboratory.

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