Vasoactive actions of local anaesthetics on human isolated umbilical veins and arteries

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1 An *in vitro* study, using helical preparations of human umbilical arteries and veins obtained from healthy women at term pregnancy, was designed to determine: (a) whether three local anaesthetics commonly utilized in obstetric anaesthesia (bupivacaine, 2-chloroprocaine, and lignocaine) can induce contraction or relaxation of resting umbilical vessels; (b) whether these agents can induce contraction or relaxation of umbilical vessels which have been previously induced to contract by a known activator, potassium chloride (KCl); and (c) the relative potency of these agents as compared to KCl.

2 The results indicate that: (a) these local anaesthetics are vasoactive on human umbilical vascular smooth muscle; (b) bupivacaine induces contraction in over 90% of the resting vessels examined, while 2-chloroprocaine consistently causes relaxation and lignocaine causes a small degree of contraction in 40% of vessels examined; (c) bupivacaine causes further contraction (or potentiation) of KCl-contracted muscle in 50% of the vessels studied, while 2-chloroprocaine and lignocaine both induce relaxation of these contracted vessels.

Introduction

Regional anaesthesia using local anaesthetics is widely used in obstetrics for labour and delivery. Foetal effects of maternal local anaesthetic administration have been under intense scrutiny for the past few years. Local anaesthetic toxicity has been reported in parturients who received regional anaesthesia and, in addition, placental transfer of these agents has been confirmed by the presence of measurable quantities in umbilical cord blood (Ralston & Shnider, 1978). Foetal and neonatal well-being has been evaluated from foetal heart rate tracings, neonatal Apgar scores, and neurobehavioural scores; and results have been compared in the presence or absence of regional anaesthesia (Ralston & Shnider, 1978). However, the direct action of local anaesthetics on the vascular smooth muscle of the umbilical cord has not been extensively studied, although this tissue is an essential component in the regulation of the umbilical-placental circulation.

Umbilical blood flow studies in man are difficult to perform and therefore largely speculative (Walsh *et al.*, 1974). Umbilical blood flow in the physiologi-

cal state is known to be pressure-dependent and relies on the resistance in the umbilical/placental unit (Walsh *et al.*, 1974). Microscopic studies of umbilical cords have demonstrated that innervation of umbilical vessels is lacking except for the intrafoetal segment (Hülsemann, 1971; Roach, 1976). In view of this, the reactivity of the umbilical vessels to vasoactive agents is of great importance, since umbilical resistance, and therefore, blood flow may be affected. Preliminary studies showed that human umbilical vessels can be used successfully in the study of vasoactive substances. Sympathomimetic amines were shown to induce responses in isolated umbilical arterial and venous strips (Somlyo *et al.*, 1965). Using umbilical vessels from human cords, varying contractile responses to 10 different vasoactive agents were demonstrated (Altura *et al.*, 1972).

Local anaesthetics are known to have vasoactive properties on a variety of vascular tissues (Covino, 1986). It is of interest to study what effect can be demonstrated on umbilical vasculature, since it is plausible that adverse physiological effects of local

anaesthetics, e.g., foetal bradycardia following paracervical block, or other clinical effects caused by maternal local anaesthetic administration, result from their direct effect on the umbilical vessels. This question was raised in a previous article which described the effects of local anaesthetics on the umbilical artery (Tuvemo & Willdeck-Lund, 1982). However, this previous study did not include the umbilical vein, which is solely responsible for delivery of oxygenated blood to the foetus, and did not include 2-chloroprocaine, a local anaesthetic which is widely used in obstetrics.

The present study, utilizing vascular smooth muscle obtained from human umbilical arteries and veins was designed to determine: (1) whether three commonly used local anaesthetics (bupivacaine, 2-chloroprocaine and lignocaine) can induce contraction or relaxation of resting umbilical vessels; (2) whether these agents can induce contraction or relaxation of umbilical vessels which have been previously induced to contract by a known activator, KCl; and (3) the relative potency of these agents compared to the latter activator.

Methods

A total of 36 umbilical cords from normal pregnancies and healthy parturients (vaginal deliveries or Caesarean sections) were utilized for this study. Approximately 10 to 25 cm segments were cut from the cords, double-clamped, and placed immediately in cold Krebs-Ringer solution buffered with sodium bicarbonate. The solution was prepared daily from a refrigerated stock solution. The composition of the working Krebs-Ringer solution, made up with deionised water, was (in mM): NaCl 118, KCl 4.7, CaCl_2 2.5, KH_2PO_4 1.2, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.2, NaHCO_3 25.0, dextrose 10, pH 7.8–8.0. The chemicals used to make up the Krebs-Ringer bicarbonate solutions, as well as the KCl were all American Chemical Society certified reagent grade. The local anaesthetics were all of a grade conventionally used for regional anaesthesia in obstetric patients: bupivacaine hydrochloride (Marcaine hydrochloride 0.25%), 2-chloroprocaine hydrochloride (Nesacaine CE 2% with sodium bisulphite 0.7 mg ml^{-1}), and lignocaine hydrochloride (Xylocaine 1%).

The arteries and veins were carefully dissected out of the cords using microdissecting instruments, while continuously bathing the cords and vessels in cold Krebs-Ringer bicarbonate buffer. The arteries and veins were cut helically and then trimmed to make vascular strips of the following dimensions: $1.5 \text{ mm} \times 20 \text{ mm}$ for arteries and $2.5 \text{ mm} \times 25 \text{ mm}$ for veins, similar to that described previously (Altura

et al., 1972). They were each suspended in a Harvard Universal Jacketed Isolated Organ Bath and kept at 37°C by a Harvard Thermocirculator. The bath apparatus consisted of a cylindrical 20 ml bath which allowed free solution inflow and outflow and bubble-through aeration. The baths were oxygenated continuously with a 95% O_2 -5% CO_2 mixture which held the pH at 7.3–7.5. The Krebs-Ringer solution used for washing the preparations between experiments was warmed to 37°C by passing through a coil warmed by the thermocirculator. One end of the muscle preparation was tied to the base of the bath while the opposite end was tied to a moveable Grass FT .03 force-displacement strain gauge connected to a 6 channel RD Beckman Instruments Dynograph. Tension on the muscle was adjusted by moving the strain gauge and was measured by pre-calibrated movement of the dynograph pen.

One artery and one vein from the same cord were utilized for each experiment. They were initially allowed 2–3 h to equilibrate in the bath under a resting tension of 2 g and were frequently washed with Krebs-Ringer bicarbonate solution (Altura *et al.*, 1972). The tissues were then tested with potassium chloride (KCl), a known contractile activator (Altura *et al.*, 1972), to confirm the integrity of the preparation and periodically throughout the experiment, as a control, to estimate vessel sensitivity and consistency. This was done by adding KCl to the bath medium and measuring the contractile force developed. The umbilical arteries and veins were exposed to each of three agents: bupivacaine, 2-chloroprocaine and lignocaine. Each agent was added to the bath medium in a cumulative manner, starting with a low concentration followed by 11 sequential additions. Ten minutes elapsed between additions to allow time for plateaus of vessel reactivity. Concentrations of the local anaesthetics and KCl were chosen such that total volumes added to the 20 ml baths never exceeded 1.0 ml. Periods of 30 min (for KCl) to 1 h (for local anaesthetics) were used to remove a drug from the bath medium by frequent washes with Krebs-Ringer solution.

Cumulative concentration-response curves were obtained according to a method previously described, where additions are made to the muscle bath to yield increasing cumulative concentrations (Van Rossum, 1963). The first phase of the experiment studied the action of the local anaesthetics on resting arteries and veins. For bupivacaine, concentrations ranged from $8.6 \mu\text{M}$ to a maximum of $430 \mu\text{M}$. For 2-chloroprocaine, the concentrations were 0.065 mM to 3.3 mM; for lignocaine, they were 0.043 mM to 2.1 mM. In addition, cumulative concentration-response curves were obtained for bupivacaine, 2-chloroprocaine and lignocaine on arteries and veins previously contracted with sub-

maximal concentrations of KCl, using the same method and concentrations described above. In each latter case, a control was done to confirm that the chosen KCl concentration produced a sustained contraction that was stable for the length of time it took to perform the local anaesthetic experiment.

For drugs which produced contraction on resting vascular muscle, results were expressed and plotted as negative log of molar contraction vs. percentage of maximal KCl-induced contraction. Where relaxation occurred, the negative log of the concentration was plotted against % relaxation of the resting 2 g tension which was applied to all tissues. For experiments on KCl-contracted muscle, negative log of concentration was plotted against % change (contraction or relaxation) of the induced contraction. The KCl concentration which reliably produced a maximal contraction in all vessels was 80 mM; submaximal concentrations used varied from 20 mM to 60 mM. The means and standard errors of the means (s.e.mean) were calculated for all induced responses. Within groups statistical significance was established by one factor analysis of variance. Unpaired one-tailed *t* test was used for comparisons between groups, and between arteries and veins, where appropriate. Statistical significance was taken as $P < 0.05$. Relative potencies of the local anaesthetics were examined using pD_2 values and maximum tension. The pD_2 value was obtained by calculating the negative logarithm of the EC_{50} , or the effective concentration which produced 50% of maximal response (Ariëns, 1964).

Drugs

Marcaïne hydrochloride 0.25% (Winthrop-Breón Laboratories, N.Y., New York, U.S.A.); Nesacaine CE 2% with sodium bisulphite 0.7 mg ml^{-1} (Astra Pharmaceutical Products, Westborough, Massachusetts, U.S.A.); Xylocaine 1% (Astra). No preservatives were present in the local anaesthetic solutions. The pH was adjusted by the manufacturer using hydrochloric acid or sodium hydroxide.

Results

Behaviour of human umbilical arterial and venous strips in vitro

As found previously, human umbilical vessel preparations showed a wide degree of variability and exhibited some spontaneous activity (Somlyo *et al.*, 1965; Altura *et al.*, 1972). For example, following the equilibration period, preparations differed from one

another with respect to baseline resting tension stability, maximal isometric tension developed in response to activator and consistency over the several hours during which the experiment was run. Occasionally a vessel would spontaneously exhibit a maximal or supramaximal contraction (as compared to a KCl-induced contraction) from which recovery to baseline was complete. Inducing a maximal sustained contraction with KCl would often reduce the occurrence of these spontaneous contractions and tend to 'stabilize' the vessel. Maximal or submaximal contractions were also seen on occasion in response to washing the preparations. As described previously (Altura *et al.*, 1972), maximal activator-induced responses were often less during the first few hours of an experiment than those seen during subsequent stimulations.

KCl-induced contraction

KCl induced contraction in all vessels examined. It was chosen as the control activator because of the magnitude of the induced response, its reproducibility between preparations in terms of concentration required to produce maximal contraction, and the ease with which the muscle preparations would recover to baseline upon washing KCl out of the bath medium. Response to KCl stimulation displayed two components described previously as 'phasic' and 'tonic' (Bolton, 1979). At low concentrations, the muscle response showed an initial peak of tension which then declined back to baseline ('phasic component'). As the concentration of KCl increased, the phasic response was followed by a sustained level of tension ('tonic component') which was sometimes less than phasic tension. The tonic contraction increased in magnitude and stability as the KCl concentration increased, until, at high KCl concentrations, a sustained maximal contraction resulted (Figure 1). In general, preparations varied widely with respect to the shape of the KCl contraction and the lowest KCl concentration required to sustain a contraction.

Response of resting umbilical arterial and venous smooth muscle to local anaesthetics

Bupivacaine induced contraction in over 90% of vessels exposed to this drug. One factor analysis of variance within groups revealed $P < 0.0001$ for veins, and $P < 0.0002$ for arteries. The pattern of contraction differed from preparation to preparation. In most cases, the contraction displayed an initial phasic upstroke followed by a tonic contraction which was lower than the peak response. This tonic phase often consisted of repeated, or 'rhythmical

Figure 1 Responses of umbilical vessels following addition of KCl. Upper tracing shows umbilical vein; lower tracing shows umbilical artery. Arrows indicate cumulative additions of KCl. Initial upstroke of contraction is not sustained until higher cumulative KCl concentrations are attained.

contractions' (Bolton, 1979) between which relaxation was incomplete, thus resulting in an increase in baseline tension. In other cases, bupivacaine caused sustained increases in baseline tension without the presence of rhythmical contractions. Developed isometric tension was measured from the lowest point

Figure 2 Responses of umbilical vein to bupivacaine. Arrows indicate cumulative additions of bupivacaine. Two phases of contractile response can be defined: initial rapid upstroke of contraction followed by sustained level of contraction.

of tension which resulted during the ten minute interval after each succeeding concentration was added (Figure 2). The concentration-response curve showed maximal venous response at a bupivacaine concentration of $220 \mu\text{M}$ (Figure 3a). At this concentration, the contraction varied from 13%–41% (mean 22.4 ± 2.6) of maximal KCl contraction depending on the particular preparation. The lowest concentration that elicited a response in some venous preparations was $22.0 \mu\text{M}$. For arterial muscle, the maximal response was obtained at $350 \mu\text{M}$ (Figure 3b) and contractions at this concen-

Figure 3 Cumulative $-\log$ concentration-isometric response curves. (a) Bupivacaine-induced contraction of resting umbilical venous smooth muscle. (b) Bupivacaine-induced contraction of resting umbilical arterial smooth muscle. Points indicate mean % of KCl maximal response. (c) 2-Chloroprocaine-induced relaxation of resting umbilical venous smooth muscle. (d) 2-Chloroprocaine-induced relaxation of resting umbilical arterial smooth muscle. Points indicate mean % relaxation of resting 2.0 g tension. In (a–d) vertical lines show s.e.mean; in (a) $n = 9$, (b) $n = 6$, (c) $n = 7$ and (d) $n = 6$ (n = number of vascular strips).

tration were from 28%–66% (mean 47 ± 6.3) of maximal KCl-induced contraction. Some arterial preparations contracted at the lowest concentration level used, $8.6 \mu\text{M}$.

In contrast to bupivacaine, 2-chloroprocaine caused relaxation of all venous and arterial preparations in a concentration-dependent manner. At the median 2-chloroprocaine concentration used in these experiments (i.e., 1.6 mM), venous muscle relaxed $11.5 \pm 1.7\%$ and arterial muscle relaxed $24.1 \pm 4.7\%$ (Figure 3c and d) as compared to the resting 2 g tension applied to all tissues. Responses were seen at the lowest concentration used, 0.065 mM , in both arteries and veins. Statistical significance was obtained using analysis of variance, with $P < 0.0001$ in veins and $P < 0.0006$ in arteries.

For lignocaine, the results obtained were less consistent, making concentration-response curves difficult to construct: 40% of the vessels examined developed increases in baseline tension; 33% had relaxation of resting 2 g tension; and 27% showed no response. In the vessels which responded, both contraction and relaxation were concentration-dependent. Maximal contractile responses were 3.9% and 9.0%, respectively, for veins and arteries; maximal relaxation was 4.5% for veins and 13.5% for arteries.

Response of KCl-contracted vascular muscle to local anaesthetics

When umbilical vessels were induced to contract with submaximal concentrations of KCl, bupivacaine demonstrated an ability to increase both the magnitude of the sustained contraction and the frequency of rhythmical contractions (Figure 4). Sustained contraction increase was observed in

Figure 4 Response of umbilical artery to bupivacaine following KCl-induced submaximal contraction. Lower panel tracing represents continuation of upper panel tracing. When umbilical vessels are induced to contract with submaximal concentrations of KCl, bupivacaine demonstrates an ability to increase the magnitude of the sustained contraction as well as the frequency of repeated contractions. Following KCl, arrows indicate cumulative additions of bupivacaine.

Figure 5 Effect of bupivacaine on KCl-contracted umbilical vessel smooth muscle (cumulative $-\log$ concentration-isometric response curves). Abscissa scales represent $-\log$ molar concentration of bupivacaine. Each tracing represents individual umbilical cord preparation. (a) Venous smooth muscle. (b) Arterial smooth muscle.

approximately 50% of the veins and arteries at a concentration which was lower than that which elicited maximal contraction on resting muscle (Figure 5). However, analysis of variance failed to reveal statistically significant sustained contraction increase ($P < 0.06$ for veins and $P < 0.37$ for arteries). At higher concentrations, relaxation of the developed tension was observed in those vessels which had initially demonstrated potentiation of the

KCl-induced submaximal contraction. The KCl-contracted vessels which did not show an increased level of contraction with bupivacaine responded with concentration-dependent relaxation of the sustained submaximal contraction. Of those vessels that showed only relaxation, analysis of variance for the veins revealed significant relaxation at $P < 0.0001$. However, no statistically significant relaxation of the arteries occurred ($P < 0.16$).

Both 2-chloroprocaine and lignocaine caused relaxation of KCl-contracted arteries and veins in a concentration-dependent manner (Figure 6). For both 2-chloroprocaine and lignocaine, significance was reached at $P < 0.0001$ for veins and arteries using within groups analysis of variance. At the median 2-chloroprocaine concentration used, i.e. 1.6 mM, $71.2 \pm 3.9\%$ relaxation of induced contraction occurred in veins and $57.8 \pm 10.5\%$ occurred in arteries (Figure 7a and b). For lignocaine, the median concentration, 1.1 mM caused $56.3 \pm 12.2\%$ relaxation in veins and $51.3 \pm 10.6\%$ relaxation in arteries (Figure 7c and d).

Relative potencies of local anaesthetic agents on human umbilical arterial and venous preparations

An analysis of the local anaesthetics relative potency based on pD_2 values and maximum tension (Table 1)

Figure 6 Response of the umbilical vein to local anaesthetics following KCl-induced submaximal contraction. Both 2-chloroprocaine (a) and lignocaine (b) caused a concentration-dependent relaxation of the KCl-induced contraction. (c) Control KCl-induced submaximal contraction, in which maximal spontaneous tension reduction at 100 min is 20%.

showed that, at resting tension, bupivacaine, which is clinically the most potent agent, also had the highest pD_2 , demonstrating high affinity for all vessels, and induced the strongest contractile responses on both

Figure 7 Relaxation of KCl-contracted umbilical vessel smooth muscle (cumulative $-\log$ concentration-isometric response curve) induced by the local anaesthetics. (a) Effect of 2-chloroprocaine on venous smooth muscle. (b) Effect of 2-chloroprocaine on arterial smooth muscle. (c) Effect of lignocaine on venous smooth muscle. (d) Effect of lignocaine on arterial smooth muscle. Points indicate mean % relaxation and vertical lines show s.e.mean. (a and d) $n = 6$, (b and c), $n = 7$ (n = number of vascular strips).

Table 1 Comparative potency of local anaesthetics on isolated umbilical vascular smooth muscle*

Agent	Preparation	Vessel	n	pD ₂ §	Maximum tension¶
Bupivacaine	Resting	Vein	9	4.1 ± 0.06	+744 ± 128
		Artery	6	4.3 ± 0.11	+1150 ± 210
	KCl-contracted	Vein	3	4.4 ± 0.09	+633 ± 117
			4	3.7 ± 0.09	-1408 ± 360
		Artery	3	4.4 ± 0.13	+243 ± 74
2-Chloroprocaine	Resting	Vein	3	3.7 ± 0.10	-1010 ± 242
		Artery	6	3.0 ± 0.06	-346 ± 51
	KCl-contracted	Vein	6	3.0 ± 0.07	-635 ± 121
		Vein	7	3.0 ± 0.06	-2733 ± 395
		Artery	7	2.9 ± 0.17	-1893 ± 300
Lignocaine	Resting	Vein	3	3.3 ± 0.23	+160 ± 21
			2	2.9 ± 0.1	-90 ± 10
			3		0†
	KCl-contracted	Artery	3	3.4 ± 0.03	+267 ± 17
			3	3.3 ± 0.19	-270 ± 38
			1		0†
		Vein	6	3.2 ± 0.05	-1588 ± 415
		Artery	6	3.2 ± 0.07	-1463 ± 234

* See text for statistical analysis.

§ pD₂ is the mean -log of EC₅₀ (the mean molar concentration required to produce 50% of the maximal response).

¶ Maximum tension is the maximal attainable response (mean ± s.e.mean) in mg; (+) contraction, (-) relaxation.

† No response (see text).

veins and arteries. One-tailed test *t* failed to show a significant difference between the pD₂ values of veins compared to arteries (*P* < 0.08). 2-Chloroprocaine, with a less potent local anaesthetic action, had considerably less potency, i.e., much lower pD₂, and displayed only relaxation. Again, pD₂ values did not differ between veins and arteries (*P* < 0.36). Lignocaine, which in some vessels showed contraction and in others relaxation, occupied an intermediate position, with no significant difference between veins and arteries (*P* < 0.35). In general, for all three agents, arteries showed more sensitivity than veins to direct action of local anaesthetics at resting tension, although statistical significance was not reached. On resting vessels, comparisons between individual agents were made utilizing unpaired one-tailed *t* test and pD₂ values. Statistical significance at *P* < 0.001 was obtained for contractile response from lignocaine as compared to bupivacaine on both veins and arteries. Significant difference in relaxation from lignocaine as compared to 2-chloroprocaine was obtained for arteries (*P* < 0.04) but not for veins (*P* < 0.23).

When the vessels were first challenged with KCl, bupivacaine showed a tendency to contract further only some of the vessels, with a higher affinity and maximum tension for the veins than the arteries, although, as noted before, statistical significance was not reached for this contractile response. With this

exception, all vessels exhibited relaxation of KCl-induced contraction when the local anaesthetics were applied. An examination of this relaxant effect to the three local anaesthetics showed the following order of potency: 2-chloroprocaine > lignocaine > bupivacaine for pD₂ values, and, as expected, 2-chloroprocaine > lignocaine > bupivacaine when maximum tension was considered. In this experimental condition, one tailed *t* test did not show significant differences between the veins and arteries for any of the agents (*P* < 0.43 for bupivacaine, *P* < 0.38 for 2-chloroprocaine, and *P* < 0.35 for lignocaine). On KCl-contracted vessels, analysis of variance was used to compare venous and arterial relaxation to all three local anaesthetic agents. Between-group variance was significantly different from within group variance, with *P* < 0.0001 for veins and *P* < 0.02 for arteries.

Discussion

To our knowledge, there have been few, and incomplete, previous experiments done to study the effect of local anaesthetics on the umbilical vasculature. Animal studies have concentrated on local anaesthetic action on vascular smooth muscle obtained

from sources other than the umbilical cord. It has been suggested that local anaesthetics may induce non-specific inhibition of vascular tone, as several of these agents were shown to cause relaxation of drug and hormone-induced contraction (Altura & Altura, 1974a). Some studies have demonstrated a contractile effect of procaine and lignocaine on animal vascular smooth muscle (Altura & Altura, 1974a) and of lignocaine on human uterine arteries (Cibils, 1976). One study, utilizing human umbilical arteries, found that prilocaine and bupivacaine produced contraction consistently (Tuvemo & Willdeck-Lund, 1982).

Since umbilical vessels lack innervation, circulating vasoactive substances, such as local anaesthetics, may interfere with the regulation of foetal blood flow by their direct action on vascular smooth muscle. This study demonstrates that local anaesthetics are vasoactive on umbilical vessel smooth muscle preparations. Contraction or relaxation of isolated vascular strips as measured in this experiment may relate to the *in vivo* physiological action of umbilical vessels in regulating blood flow to the foetus.

The concentrations of local anaesthetics used in this study were slightly higher than maternal plasma concentrations measured following administration of local anaesthetics for labour or Caesarean section (Ralston & Shnider, 1978). Rapid *in vivo* hydrolysis of 2-chloroprocaine reduces absorbed concentrations to nanogram levels. High maternal protein binding is responsible for reduced placental transfer of bupivacaine. However, inadvertent intravascular injection of local anaesthetics leads to plasma concentrations well in excess of those that were measured under controlled circumstances (Moore *et al.*, 1980). Furthermore, the intact microcirculation is 10,000 to 100,000 times more sensitive to drugs than are some isolated large vessels (Altura & Altura, 1974b). Therefore, although the drug concentration to which the *in vitro* isolated smooth muscle is exposed appears large; in fact, *in vivo*, the microcirculation will respond to many times lower concentrations than will the rest of the vasculature.

In the concentrations used for our studies, the only effect of 2-chloroprocaine on umbilical vasculature was relaxation. This was seen in both resting and contracted vessels and was consistently obtained. One could, therefore, postulate that even in the event of high maternal concentrations of 2-chloroprocaine, umbilical blood flow would not be reduced based on a direct action of this agent on the umbilical vasculature.

No consistent or marked response of resting umbilical vessels to lignocaine was seen in our study, although relaxation of KCl-contracted muscle was reproducible. Earlier accounts of depressed neurobehavioural scores in neonates whose mothers had

received epidural anaesthesia with lignocaine have been refuted (Abboud *et al.*, 1983b; Kileff *et al.*, 1984). The aetiology of foetal bradycardia in paracervical block with lignocaine is still unknown, and it is possible that there is some relationship between this complication and the vasoactive properties of lignocaine. The results of our study did not demonstrate conclusively that lignocaine causes adverse foetal effects by a direct action on umbilical cord vascular smooth muscle.

Bupivacaine consistently produced contractions in resting umbilical arteries and veins. The magnitude of maximal sustained contraction ranged from 13%–41% in venous muscle and from 28%–66% in arterial muscle as compared to maximal KCl-induced contraction. In addition, umbilical vascular smooth muscle in many cases displayed contractile peaks which were up to four times higher than the level of sustained tension (Figure 2). Bupivacaine also displayed an ability to increase the tension of a KCl-induced contraction.

These findings are of particular interest in view of reports implicating bupivacaine in adverse maternal and foetal effects. Although favourable foetal outcome as assessed by Apgar scores, acid-base status, and neurobehavioural scores following regional anaesthesia with bupivacaine has been obtained (Scanlon *et al.*, 1976; Abboud *et al.*, 1983a; Kileff *et al.*, 1984), questions have been raised concerning its safety in uncomplicated regional anaesthesia. Data have shown a significantly increased incidence of foetal bradycardia following epidural anaesthesia with bupivacaine as compared to epidural anaesthesia with lignocaine or 2-chloroprocaine; and following paracervical block with bupivacaine (Teramo, 1969; Abboud *et al.*, 1982). There are accounts of bupivacaine-induced maternal central nervous system and cardiovascular toxicity, with the latter accounting for cases of refractory cardiovascular collapse. In these cases, inadvertent intravascular injection is the presumed aetiological factor. Poor foetal outcome has been attributed to adverse maternal cardiovascular effects, although foetal asphyxia has been noted in cases where bupivacaine induced maternal seizures in the absence of cardiovascular depression (Albright, 1985).

Our study suggests that intravascular injection of intended epidural bupivacaine may lead to plasma concentrations sufficiently high to cause umbilical vessel contraction with adverse foetal effects resulting via this mechanism. Although plasma bupivacaine concentrations are not reported in cases of severe maternal toxicity, there have been published case reports which include plasma bupivacaine concentrations following presumed inadvertent intravascular injection in surgical patients (Moore *et al.*,

1980). Experimental and human volunteer studies using concentrations which are considered to be in excess of bupivacaine toxic concentrations are similar to *in vitro* concentrations used in our study; e.g., contractile responses in some umbilical arteries at $8.6 \mu\text{M}$ ($2.5 \mu\text{g ml}^{-1}$) and in some veins at $22.0 \mu\text{M}$ ($6.25 \mu\text{g ml}^{-1}$) (Mather *et al.*, 1971; Denson *et al.*, 1984; Kotelko *et al.*, 1984; Sage *et al.*, 1985; Kasten, 1986).

Caution must be exercised when one extrapolates findings of an *in vitro* study to an *in vivo* situation. However, the data presented here suggest that bupivacaine may have clinically significant adverse effects based on its ability to contract umbilical vascular smooth muscle. 2-Chloroprocaine showed only

smooth muscle relaxation, and the effect of lignocaine was not predictable, with only a small degree of contraction or relaxation seen in some preparations. In view of these findings, it seems prudent to explore further this avenue of research. The importance of the umbilical vasculature to foetal well-being suggests that agents used in pregnancy should be more closely examined with respect to their structure-activity relationships on the human umbilical-placental vasculature.

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