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COMMUNICATIONS

Postoperative course of plasma protein binding of lignocaine, ropivacaine and bupivacaine in sheep

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Abstract—The plasma protein binding of the 2,6-xylidide local anaesthetic agents lignocaine, ropivacaine and bupivacaine enantiomers was determined by equilibrium dialysis in plasma obtained from chronically catheterized sheep before and up to 21 days after surgery. Three concentrations (1, 5 and 10 mg L^{-1}), were used for each agent. Concentration-dependent binding was evident for each agent throughout the study period. *R*(+)-Bupivacaine was more extensively bound than *S*(−)-bupivacaine at the higher concentrations. Compared with pre-surgery, binding of each agent was less on the first postoperative day but did not differ significantly from days 8 to 21.

Information about drug binding in plasma can be important in the interpretation of blood level and pharmacokinetic data. The main proteins associated with drug binding in plasma are albumin, α_1-acid glycoprotein (AAG) and the lipoproteins, with albumin being the protein principally responsible for the binding of acidic or anionic drugs, and AAG principally responsible for the binding of basic or cationic drugs. Variations in plasma concentrations of these binding proteins, in particular the acute phase reactant AAG, occur during a variety of pathophysiological states, for example, in disease states (Tillement et al 1978), trauma (Edwards et al 1982) and surgery (Aronsen et al 1972). That the disposition of lignocaine is affected by AAG levels has been demonstrated after myocardial infarction (Routledge et al 1981; Holley et al 1984). Variation in protein binding can thus play a major role, not only in determining central nervous system effects of a drug such as bupivacaine (Denson et al 1984a), but also in its distribution and elimination.

Our laboratory has made extensive use of a chronically catheterized conscious sheep preparation (Runciman et al 1984) to investigate, amongst other things, the disposition of drugs commonly used in anaesthesia. In particular, the 2,6-xylidide local anaesthetic agents lignocaine, ropivacaine and bupivacaine have been investigated with regard to their total body and regional kinetics (Mather et al 1986; Rutten et al 1990, 1991). The sheep preparation requires surgical procedures for the implantation of intravenous cannulae with experiments generally taking place between one and three weeks after surgery. Despite the potential of altered drug protein binding associated with surgery, a post-surgical protein binding profile has not previously been reported.

This study reports the influence of surgery on the protein binding of local anaesthetic agents. The concentration range studied encompassed that which occurred during the disposition studies. Bupivacaine, although studied as the clinically used racemate, was assayed enantioselectively.

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Materials and methods

Animals. Adult Merino ewes, aged between 1 and 2 years, were acclimatized in metabolic cages before surgery in an animal room for a minimum of 3 days before use. Food and water were readily available throughout the acclimatization and experimental periods. A detailed description of the general methods and materials used for the animal preparation has been reported (Runciman et al 1984; Rutten et al 1990); the methods specific to the present study are described here briefly. After anaesthesia was induced (intravenous sodium thiopentone 20 mg kg^{-1}) and maintained (1–1·5% halothane, 40% oxygen, balance nitrogen) the abdominal aorta and renal artery were exposed, as if to place the usual cannulae. The skin incision was then closed without the cannulae being placed in either vessel. After surgical exposure of the vessels in the neck, two arterial catheters were placed with their tips approximately 2 cm above the aortic valve. Additional catheters were placed in the right jugular vein, one with its tip in the right atrium, the other, a flow-directed thermodilution catheter, was placed with its tip in the pulmonary artery. Three sheep were thus prepared. For each animal, the time from induction of anaesthesia to recovery was approximately 3 to 4 h. A single dose of methadone (10 mg) was administered for postoperative analgesia.

Blood sampling. From each animal, arterial blood (30 mL) was collected in heparinized tubes (3 tubes, 125 int. units per tube) immediately before induction of anaesthesia and then 1, 2, 4, 8, 12, 17, and 21 days after surgery. To preclude any effects of diurnal variation all blood samples were taken at similar times (0800 h) on each day. The blood was centrifuged (3000 rev min^{-1} for 10 min), the plasma harvested and portions used for the determination of protein binding of each drug by the method of equilibrium dialysis. An additional 10 mL was taken at selected times throughout the study period to test for bacteraemia.

Equilibrium dialysis. The plasma protein binding of lignocaine, ropivacaine and bupivacaine enantiomers was determined by equilibrium dialysis in Teflon cells in a Dianorm apparatus. The compartments were separated by a semi-permeable membrane with molecular weight cut-off of 12 000–14 000 Da (Spectropor-2-cellulose, Spectrum Medical Industries, CA, USA). Before use, each membrane was soaked in distilled water for 4 h, then overnight in isotonic phosphate buffer (Na_2HPO_4, 0·074 M, pH 7·4). Blank plasma (1 mL) was introduced into one compartment of the Teflon cell and an equal volume of isotonic phosphate buffer (pH 7·4, freshly adjusted with phosphoric acid), containing drug at a concentration of 1, 5 or 10 mg L^{-1} (as the HCl salt) into the other compartment. For each sheep, drug was added to 30 mL buffer which was used as a pool from which 1 mL was taken on each occasion. This ensured that the same concentra-

tion was used throughout the study. For each drug of intermediate concentration, duplicate dialysis procedures were performed. Thus a total of 4 cells was used for each drug on each day. The cells were rotated (16 rotations min^{-1}) for 6 h in a water bath maintained at 37°C. Previous studies had confirmed equilibrium within this period (Nancarrow et al 1987). At the completion of dialysis, plasma and buffer were collected in preweighed containers and stored at −20°C until assay. Post-dialysis plasma pH was not measured as this has been shown not to vary significantly during rapid equilibrium dialysis (Ridd 1982).

Determination of drug concentrations. Lignocaine and ropivacaine concentrations in plasma and buffer were determined by gas-liquid chromatography with nitrogen-selective detection after solvent extraction using methods previously described (Nancarrow et al 1987; Rutten et al 1990). The enantiomers of bupivacaine in plasma and buffer were determined by HPLC after solvent extraction also using methods previously described (Rutten et al 1991). Coefficients of variation of the assays for lignocaine, ropivacaine and for *R*(+)- and *S*(−)-bupivacaine were 2·0, 2·2, 5·0 and 5·9%, respectively. The standard curves were linear and passed through the origin. The limit of detection of lignocaine and ropivacaine was approximately 5 ng mL^{-1} and that for the enantiomers of bupivacaine was 10 ng mL^{-1}.

Data and statistical analysis. Percentage bound was calculated from the ratio of the drug concentration difference in plasma and buffer to that in plasma, multiplied by 100. Differences in the % of drug unbound between times (day 8–21) for each drug and concentration were examined by a single factor repeated measures analysis of variance. For each animal, evidence of enantioselective binding of the enantiomers of bupivacaine was examined by comparing the differences in mean values of the percentage unbound of *R*(+)- and *S*(−)-bupivacaine, using Student's *t*-test for paired data at each concentration. All statistical tests were two tailed and $P<0·05$ was considered significant.

Results

The mean absolute percentage difference in percentage drug unbound calculated from the duplicate dialysis procedures on each day for each drug are presented in Table 1. On average, the percentage unbound differed by 10–13% between duplicate determinations.

The time course of the percentage unbound for each drug at each concentration is shown in Fig. 1. Decreased binding occurred with increasing concentrations for each drug. The binding was always lowest on the first postoperative day and increased toward pre-operative values on days 2 to 4. There was no significant difference in the percentage unbound from days 8 to 21 at any concentration of any drug. There were significant differences in the percentages unbound of *R*(+)- and *S*(−)-bupivacaine at the higher concentrations (Table 2).

Table 1. Absolute mean percentage differences in the percentage unbound determined from the duplicate dialysis procedures for each drug. As the values were not different for each sheep, these have been combined.

Drug	Absolute mean difference (%) (±s.d.)	n
Lignocaine	11±10	22
Ropivacaine	10±7	24
R(+)-Bupivacaine	13±9	23
S(−)-Bupivacaine	13±10	23

n = total number of duplicate dialysis procedures performed.

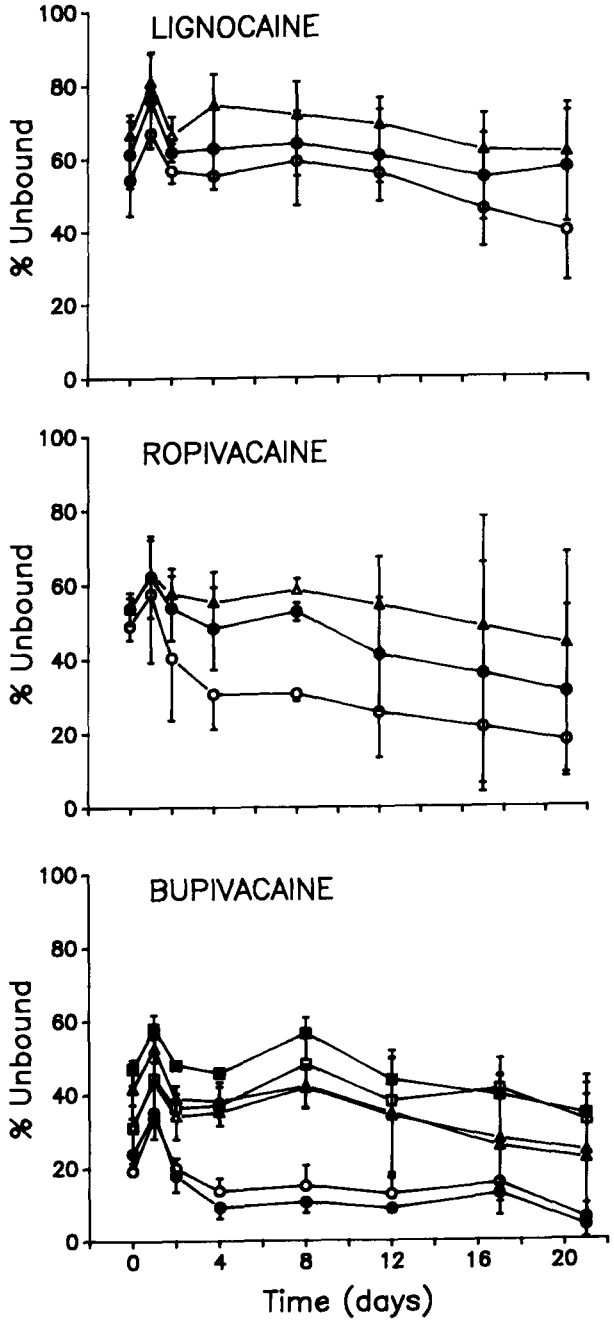

FIG. 1. Plasma binding of lignocaine, ropivacaine and bupivacaine following surgery. Surgery was performed immediately after collecting blood for analysis on day 0. Values were determined ex-vivo and are shown as the mean±s.d. Pre-dialysis plasma concentrations ranged from 0·5 to 10·0 mg L^{-1}. Lignocaine, ○ 1·0, ● 5·0, △ 10·0 mg L^{-1}; ropivacaine, ○ 1·0, ● 5·0, △ 10·0 mg L^{-1}; bupivacaine, ○ 0·5, △ 2·5, □ 5·0 mg L^{-1} *R*(+)-, ● 0·5, ▲ 2·5, ■ 5·0 mg L^{-1} *S*(−)-.

Discussion

The binding of the 2,6-xylidide local anaesthetic agents is dominated by a high affinity low capacity association with AAG

Table 2. Mean percentage of the bupivacaine enantiomers at each concentration for each animal.

Enantiomer concn (mg L^{-1})	Sheep	Mean % unbound[a] $R(+)$-	Mean % unbound[a] $S(-)$-	P[b]
0·5	1	13	15	
	2	19	17	0·55
	3	20	15	
2·5	1	28	30	
	2	34	38	0·036
	3	40	43	
5·0	1	37	43	
	2	37	44	0·025
	3	42	53	

[a] Mean of all the 7–8 measurements in each sheep. [b] Significance from Student's t-test for paired data.

and low affinity high capacity association with albumin (Mather & Thomas 1978; Denson et al 1984b; Tucker 1990). Surgery, with its associated changes in physiology and metabolism, results in a decrease in plasma albumin concentration and a marked increase in AAG, the level of which remains elevated for several days. For example, increased binding of quinidine was shown to parallel a rise in AAG in postoperative patients (Fremstad et al 1976) and this remained elevated for as long as 3 weeks after surgery. Similarly, Davies et al (1988) reported that significant changes in the plasma binding of lignocaine, quinidine and propranolol were associated with marked fluctuations of plasma proteins during the first 5 days after cardiac surgery. The results of the present study have indicated that, in the sheep, a major surgical procedure had no effect on the extent of plasma protein binding of the local anaesthetic agents during 8–21 days post-surgery. That the animals remained in good health throughout the study period was borne out by the absence of bacteraemia and the absence of large fluctuations in the haemodynamic variables, temperature, heart rate and cardiac output. Verification of this was deemed essential as disease state has been shown to alter the level of plasma proteins (Tillement et al 1978).

Examination of Fig. 1 indicates that there is an increase in the percentage unbound for all drugs associated with the first postoperative day. This may be explained by a decrease in concentration of proteins (which is contrary to expectation) or to changes in free fatty acid (FFA) levels. It is unlikely that decreased protein levels could be attributed to massive fluid shifts as there was minimal blood loss associated with the surgery. It is more likely that an increase in FFA levels, with consequent displacement of the drugs from binding sites, could have contributed to the decrease in observed binding. An increase in FFA levels has been documented to occur temporarily after physiologic stress, the effect being mediated by catecholaminergic activation of lipoprotein lipases (Riemersma et al 1974). In addition, heparin is known to raise the level of FFA as a result of increased lipoprotein lipase activity (Fraser et al 1961). The catheters used in these animals were kept patent by heparinized (5 int. units mL^{-1}) saline. During their placement frequent flushing would have delivered more heparin than the low level (2 mL h^{-1}) flow used to maintain their patency.

Changes in binding with increasing drug concentrations were found as is now well-known for such drugs (Tucker 1990). As in human plasma, two classes of binding sites have been shown in sheep plasma (Coyle et al 1984). However, there is some evidence that there may be differences in the binding isotherms for basic drugs on sheep and human proteins (Coyle et al 1984; Hill et al 1986). We are not aware of a radial immunodiffusion assay specific to sheep AAG that could be used to investigate this issue further. The present study, however, indicates that the high affinity-low capacity sites become saturated at lower concentrations than in man.

In contrast to other reports from the individual enantiomers of bupivacaine (Tucker et al 1970; Mather 1991) higher binding of the $R(+)$- than the $S(-)$-enantiomer was found. In the present study racemate was added to the buffer sample and enantioselective assay was used. This allowed standard curves to be constructed for each enantiomer from the same stock solution of the racemate. This design eliminates a number of potential sources of error (e.g. separate weighings, separate equilibrations, and assay of different samples for each enantiomer) when the enantiomers are studied separately, and hence is likely to provide greater power to detect small differences in binding. It is not surprising that stereoselectivity in binding occurs since proteins themselves are chiral compounds. Stereoselective binding has been documented for the enantiomers of other basic drugs including verapamil (Gross et al 1988), propranolol (Walle et al 1988), and mepivacaine (Mather 1991).

Thus, it can be concluded that although concentration-dependent and enantioselective plasma protein binding was exhibited, no difference in the extent of protein binding of these basic drugs was observed over the 8–21 day postoperative period that would influence the interpretation of pharmacokinetic data.

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Stimulation of faecal excretion in rats by α_2-adrenergic antagonists

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Abstract—The effects of several α-adrenoceptor antagonists on faecal output and water content in rats were investigated. Fed rats were treated either subcutaneously (s.c.) or orally with phentolamine, idazoxan, yohimbine, 1-(2-pyrimidinyl) piperazine (PmP) or prazosin. Drug potencies were compared on the basis of the dose inducing excretion of 1 g dry weight of faeces (AD1) by rats that do not normally excrete any faecal pellet during the observation time. The α_2-antagonist, idazoxan (AD1 = 0·25 mg kg^{-1}, s.c.) was approximately 2·5, 4 and 8 times more potent than PmP, phentolamine and yohimbine in promoting faecal excretion. Prazosin, an α_1-antagonist with putative affinity for the α_{2B}-receptor subtype, was the least effective (AD1 > 5 mg kg^{-1}, s.c.). The same compounds also increased the water content of faeces and had similar potencies by the oral route. Both clonidine (0·15 mg kg^{-1}, s.c.) and atropine (0·2 mg kg^{-1}, s.c.) significantly prevented the effects of all antagonists on faecal excretion. The present results are consistent with the view that rat colon is under tonic inhibitory control of prejunctional α_2-adrenergic receptors, whose blockage by specific antagonists induces faecal excretion. The α_{2A}-receptor subtype appears to be the most likely candidate for controlling faecal excretion through inhibition of acetylcholine release.

Coordination of motor activity and regulation of gastrointestinal fluid transport are important physiological functions of the enteric nervous system. In this context, the α_2-adrenoceptors were found to play a prominent role as modulators of intestinal propulsion and fluid secretion (DiJoseph et al 1984; Crema & De Ponti 1989). The α_2-agonist clonidine has been reported to be a potent antidiarrhoeal drug (Lal et al 1981; Megens & Niemegeers 1984) and an inhibitor of defaecation under normal (Doherty & Hancock 1983) and stressful conditions (Laverty & Taylor 1969). On the other hand, the α_2-antagonist yohimbine has been shown to facilitate in-vitro release of acetylcholine and to increase the peristaltic reflex (Marcoli et al 1987).

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The aim of the present study was to evaluate the functional importance of the α_2-adrenergic system in modulating faecal excretion by conscious rats, in view of the now recognized hetereogeneity of α_2-adrenergic receptors and, especially the recently postulated existence of separate α_{2A}- and α_{2B}-adrenoceptor subtypes (Bylund 1988; Young et al 1989; Gobbi et al 1990).

Materials and methods

Male Crl:CD(SD)BR rats (Charles River, Italy), 220–250 g, were kept individually in grid-floor cages, with food and water freely available. At 0800 h food was withdrawn and 3 h later the rats were given the test compounds subcutaneously (s. c.) or orally (p. o.). Drugs were dissolved in 0·9% NaCl (saline) or distilled water (except prazosin, which was dissolved in a 10% aqueous solution of polyethylene-glycol) and administered in a volume of 2 mL kg^{-1}.

Treatments were assigned from random tables, each group consisting of eight animals. Immediately after treatment, gentle thumb pressure was applied to the perianal region to expel faecal pellets from the rectum. The pellets discharged during the next 90 min (s.c. treatment) or 210 min (p.o. treatment) were collected and weighed immediately (wet weight) and after drying (10 h at 50°C) to constant weight (dry weight). The doses inducing 1 g (dry weight) faecal excretion (AD1) were extrapolated from log-dose response-lines (Finney 1964).

Any action on secretion or reabsorption of fluids was assessed from the ratio of wet to dry faecal weights. The normal ratio was calculated from the faeces excreted throughout the 2 h preceding treatment, since control rats generally did not defecate during the observation period (90–210 min).

Statistical analysis of the effects of tested compounds on faecal excretion was based on the Dunnett test (Dunnett 1955).

The following drugs were purchased from commercial sources