Bupivacaine binding to plasma protein fractions

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Amide-type local anaesthetic agents (lignocaine, mepivacaine, bupivacaine, etidocaine) are frequently used to provide analgesia in labour. A concentration differential between maternal and foetal plasma is consistently observed in samples taken at delivery. Differences in binding to proteins in maternal and foetal plasma have been suggested as being responsible for the concentration difference observed for some local anaesthetics at delivery (Tucker, Boyes & others, 1970: Mather, Long & Thomas, 1971). by filtration. Equilibrium dialysis and analyses were performed according to the previously described technique (Mather & others, 1971). Results of binding studies are reported in Table 1 along with previously reported values for whole plasma and albumin for comparison. A coefficient of variation of 8·5% (n=3) was typical over the range of concentrations and systems examined.

It is obvious that there is a marked difference in bupivacaine binding capacity between the different

Table 1. *Binding of bupivacaine to plasma proteins.*

Total bupivacaine concn. (nominal μg ml^{-1})	Adult pooled* plasma (% bound)	Albumin* 48 g litre^{-1} (% bound)	α_1-Lipoprotein 3·1 g litre^{-1} (IV-5)** (% bound)	α_2-Mucoptotein 4·8 g litre^{-1} (IV-6)** (% bound)	β_2-Globulin 8 g litre^{-1} (III-I)** (% bound)	γ-Globulin 7·4 g litre^{-1} (II)** (% bound)
0·04	N.D.	35·1	N.D.	N.D.	0	37·1
0·10	92·1	34·2	N.D.	19·7	0	33·9
0·20	85·5	N.D.	86·1	18·8	0	N.D.
0·25	83·5	33·5	79·2	15·5	0	18·3
0·50	81·0	33·0	45·2	16·0	0	14·8
5	77·6	31·0	22·0	17·4	0	13·5
20	73·8	N.D.	N.D.	9·0	0	8·3
50	74·2	28·7	14·7	7·0	0	5·3

* Previously reported values from Mather & others (1971).
** Cohn Designation.
N.D. = Not Determined.

Using bupivacaine as a model agent, Thomas, Long & others (1976) reported that there was no difference in the unbound drug concentration in paired maternal and foetal plasma samples taken at delivery. Hence, nett differences in the total drug concentrations at delivery could be attributed to differences in protein binding between maternal and foetal plasma. Bupivacaine is 98–75% bound at clinically-occurring concentrations in maternal plasma. At the same concentrations, it is 90–0% bound in cord blood plasma (Thomas & others, 1976). Mather & others (1971) reported that binding to human albumin was 35–33% over the clinically occurring range of concentrations (0·1–0·5 μg ml^{-1}). The present report concerns the binding of bupivacaine to purified proteins from human adult plasma.

Purified fractions of adult human plasma proteins (gift from Commonwealth Serum Laboratories, Melbourne, Australia) were prepared in pH 7·4 buffer (Sorensen phosphate, M/15) at approximately physiological concentrations (Table 1) and then sterilized

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proteins. In contrast to the other proteins, α_1-lipoprotein demonstrated high affinity, but low capacity, for bupivacaine. This would appear important in the development of associations at low concentrations. No binding at all could be demonstrated with the β_2-globulins. In all cases, binding was concentration-dependent. Bupivacaine is a very lipophilic drug (Tucker, 1975). Therefore it is not surprising that it should be associated with lipoproteins. Further, there is a relative lack of the α_1-lipoproteins in foetal plasma (Diem & Lentzner, 1970). Hence it is probable that this contributes to the binding difference which results in the concentration difference observed at delivery.

Early suspicions (Olsen, 1973) concerning the role of proteins other than albumin being responsible for binding of basic drugs have been recently reinforced (Judis, 1977; Vallner, 1977; Vallner & Chen, 1977). This information, coupled with the now well-documented differences in maternal and foetal plasma protein binding is a necessary pre-requisite to the correct interpretation of the relations between plasma concentrations, disposition and pharmacological activity of the clinically-important local anaesthetic agents (Tucker, 1975).

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'Antagonist'-precipitated withdrawal in the rat after chronic Δ^9-tetrahydrocannabinol treatment

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It is well documented that tolerance develops in man (Jones & Benowitz, 1976) and laboratory animals (Paton, 1975) to many of the effects of cannabis extracts or Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive constituent of cannabis. Evidence for dependence on cannabis or Δ^9-THC preparations is less impressive, with most studies reporting lack of abstinence syndromes after chronic treatment (Leite & Carlini, 1974; Snyder, 1971). However, a few investigations have revealed withdrawal signs following the cessation of chronic administration of Δ^9-THC in the monkey (Kaymakcalan, 1972) and man (Jones, 1971; Jones & Benowitz, 1976), and one study has reported naloxone-precipitated withdrawal in rats (Hirschhorn & Rosecrans, 1974). The earlier literature may have discounted a cannabis abstinence syndrome in animals and man because of the inappropriate comparisons made with the more striking phenomena associated with opiate withdrawal. In addition, unlike experience with the opiates, abstinence symptoms to cannabis have not been precipitated because a specific antagonist has not been identified. Previous work has implicated the involvement of serotoninergic mechanisms in several of the actions of Δ^9-THC (Sofia, Dixit & Barry, 1971; Ho & Johnson, 1976; Taylor & Fennessy, 1977). Clomipramine (chlorimipramine), a potent inhibitor of 5-HT uptake (Lidbrink, Jonsson & Fuxe, 1971), appears to antagonize the Δ^9-THC-induced hypothermia and changes in brain monoamines of the rat (Fennessy & Taylor, 1978). We undertook to determine whether rats, chronically treated with Δ^9-THC, exhibit changes in behaviour induced by cessation of treatment or by injections of clomipramine. We observed that administration of clomipramine, but not cessation of Δ^9-THC, produced quantifiable behavioural changes which may indicate the precipitation of a withdrawal response.

All experiments were conducted in a room at an ambient temperature of 21 ± 2° and a 12 h light–dark cycle. Cannulae were implanted into the external jugular veins of individually-caged male Wistar rats, 240–280 g (Fennessy & Taylor, 1977). After recovery for 48 h, rats were divided into two groups of ten, each group receiving intravenous injections of either Δ^9-THC or its vehicle, polyvinylpyrrolidone (PVP, Fenimore & Loy, 1971), twice daily for 10 days. The following schedule of doses of Δ^9-THC and PVP was used:

	Dose (mg kg^{-1})			
	Δ^9-THC		PVP	
Day	a.m.	p.m.	a.m.	p.m.
1	2	2	40	40
2–4	4	4	80	80
5	4	6	80	120
6–10	6	6	120	120

On day 11, each group was further divided into two groups of 5 rats. Rats from all groups were placed individually in 10 litre opaque plastic buckets and allowed to acclimatize for 30 min. One group of the Δ^9-THC-treated and one group of the PVP-treated rats were then injected intraperitoneally with clomipramine HCl, 15 mg kg^{-1}. The other two groups were given injections of normal saline. Overt behaviour, such as jumps, kicks, wet shakes and writhes, were recorded for the next 30 min. The animals were then returned to their home cage and their behaviour observed for the next 13 days. Body temperature was recorded by means of a thermistor probe inserted 6–7 cm into the colon 30 min before and 30 min, 4 and 8 h after the injection of clomipramine or saline, and once daily for the next 13 days. Body weight was measured twice daily throughout the 23 day experi-

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