availability of haem for synthesis of the haemoprotein and a decrease in the regulatory 'pool' of haem.

The activity of microsomal haem oxygenase was also increased by lead pretreatment. It is therefore concluded that the depression in the microsomal mixed function oxidase system in lead intoxication is the result of depressed haem synthesis and also possibly by an increase in haem degradation.

References

ALVARES, A.P., LEIGH, S., COHN, J. & KAPPAS, A. (1972). Lead and methyl mercury: effects of acute exposure on cytochrome P-450 and mixed function oxidase system in the liver. *J. Exp. Med.*, **135**, 1406–1409.

SCOPPA, P., ROUMENGOUS, M. & PENNING, W. (1973). Hepatic drug metabolizing activity in lead poisoned rats. *Experientia*, **29/8**, 970–972.

The metabolism of bupivacaine in the rat

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Bupivacaine (Marcain, *N*-*n*-butylpipecolylxylidine) is a long-acting local anaesthetic widely used for lumbar epidural anaesthesia in childbirth. It is almost completely metabolized in human volunteers, mothers in childbirth and by their babies (Caldwell, Moffatt, Smith, Lieberman, Cawston & Beard, 1976) but little is known of the pathways of metabolism. Accordingly we have investigated the fate of this drug in the rat as a preliminary to human studies.

[^{14}C]-Bupivacaine hydrochloride, labelled in the carbonyl group, was synthesized, administered to female Wistar albino rats (30 mg/kg; 2.5 μCi/animal) by intraperitoneal injection, and their urine and faeces collected for four days. The excretion of [^{14}C] was monitored by liquid scintillation counting, and 74% of the dose was recovered (33% urine: 41% faeces). The bulk of the [^{14}C] excreted (urine 27%, faeces 29%) appeared on the first day.

Urinary metabolites were examined by thin-layer chromatography followed by radiochromatogram scanning, reverse isotope dilution, gas liquid chromatography, gas chromatography-mass spectrometry (g.c.-m.s.) and u.v. spectrometry. Five urinary metabolites were identified, together with two unknowns, and their identities and quantities are given in Table 1. The major routes of metabolism of bupivacaine in the rat involve hydroxylation of the aromatic ring to yield two isomeric phenolic metabolites, 3′- and 4′-hydroxybupivacaine, excreted free and as glucuronic acid conjugates, the ratio of free to conjugated material being about 0.25 in both cases. Standard compounds were not available, and the g.c.-m.s. properties of these metabolites do not permit unequivocal assignment of the position of the hydroxyl group and the assignment of these metabolites is by analogy with those of local anaesthetics of similar structure (see Dring, 1976). *N*-dealkylation to yield desbutylbupivacaine was a minor pathway, as was hydrolysis of the amide bond giving pipecolic acid. Two unknown minor metabolites were also separated but not characterized, and a small amount of bupivacaine was excreted unchanged.

The fate of bupivacaine in the rat is similar to that of other xylidide local anaesthetics, such as lignocaine and mepivacaine, especially in the predominance of aromatic hydroxylation (see Dring, 1976). However, the extent of *N*-dealkylation and amide hydrolysis is much lower than in the case of lignocaine, probably due to the size of the *N*-*n*-butyl substituent and to steric factors associated with the pipecolic acid moiety.

Table 1 Urinary metabolites of bupivacaine in the rat

Compound	*% Urinary ^{14}C as that compound*
Bupivacaine	3.4
Desbutylbupivacaine	1.1
3′-Hydroxybupivacaine	
free	8.9
glucuronide	36.4
4′-Hydroxybupivacaine	
free	5.6
glucuronide	22.7
Pipecolic acid	6.0
Unknown 1 (neutral)	2.2
Unknown 2 (acidic)	13.1

0–24 h urine of rats dosed with [^{14}C]-bupivacaine was analysed as described. Figures are the means of 3 animals.

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References

CALDWELL, J., MOFFATT, J.R., SMITH, R.L., LIEBERMAN, B.A., CAWSTON, M.O. & BEARD, R.W. (1976). Pharmacokinetics of bupivacaine administered epidurally during childbrith. *Brit. J. Clin. Pharmacol.*, 3, 956–957P.

DRING, L.G. (1976). Species variations in pre-conjugation reactions of non-primate mammals in *Drug Metabolism—from Microbe to Man*, eds. Parke, D.V. & Smith, R.L. Taylor & Francis, London. pp. 281–298.

Cardiovascular actions of prostacyclin (PGI_2) in chloralose anaesthetized dogs

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Prostacyclin (PGI_2 or PGX) is an unstable intermediate of arachidonic acid metabolism, which strongly inhibits aggregation of human platelets (Moncada, Gryglewski, Bunting & Vane, 1976) and relaxes several isolated vascular preparations (Bunting, Gryglewski, Moncada & Vane, 1976; Dusting, Moncada & Vane, 1977). Prostacyclin is the predominant active metabolite of the cyclic endoperoxides in all vascular tissues studied, including bovine coronary arteries (Dusting *et al.*, 1977). We have now measured the cardiovascular effects of prostacyclin in anaesthetized open-chest dogs.

Anaesthesia was induced with thiopentone (20–25 mg/kg) and maintained with chloralose (50 mg/kg, i.v.) supplemented (5 mg/kg i.v.) as required. The dogs were artificially ventilated; arterial Po_2 was maintained above 100 mmHg and Pco_2 in the range 28–40 mmHg. Electromagnetic flow probes (Statham Instruments Inc.) were fitted to the ascending aorta (5 dogs) and to the left circumflex artery (8 others). Cardiovascular parameters were recorded as previously described (Hughes, 1971). Mean coronary flow (over 4 s intervals) and coronary vascular resistance were also computed. Drugs were infused into the right femoral vein, into a cannula in the left atrial appendage, and in some experiments via a fine catheter in the left circumflex artery distal to the flow probe.

Intravenous infusion of prostacyclin for 3 min (50–1,000 ng kg^{-1} min^{-1}) caused dose-dependent decreases in systemic blood pressure, total peripheral resistance, and coronary vascular resistance and moderate increases in stroke volume and cardiac output (maximum increase $72 \pm 20\%$, mean $\pm$ s.e. mean). Heart rate changes were variable. The stable metabolite of prostacyclin, 6-oxo-$PGF_{1\alpha}$ had no effect (up to 10 μg kg^{-1} min^{-1}).

The cardiovascular effects of prostacyclin were similar after intravenous or left atrial infusions. Whereas prostaglandin E_1 (left atrial infusions) was slightly more potent than prostacyclin, it was about one tenth as potent when given intravenously.

Intravenous infusion of prostacyclin (50–500 ng kg^{-1} min^{-1}) increased peak phasic coronary flow only at the highest infusion rate. However, mean coronary flow did not change significantly at any infusion rate although coronary vascular resistance was substantially reduced. Direct injection of prostacyclin (50–1000 ng) into the left circumflex artery increased both phasic and mean coronary flow and decreased coronary vascular resistance (for 1–4 min) without any change in systemic blood pressure or heart rate. Similar effects with PGE_1 were more persistent (up to 8 min) whereas PGE_2 was less potent.

After indomethacin (5 mg/kg i.v.: 4 dogs) or sodium meclofenamate (2 mg/kg i.v.: 2 dogs) there was a slow increase in systemic blood pressure and coronary vascular resistance, and a reduced phasic and mean coronary flow. Indomethacin and meclofenamate potentiated the coronary dilator effects of the smaller intravenous infusions of prostacyclin but did not alter the hypotensive effects. Thus, the sensitivity of the coronary vascular bed *in vivo* is enhanced when endogenous biosynthesis of prostaglandin-like substances is inhibitied.

References

BUNTING, S., GRYGLEWSKI, R., MONCADA, S. & VANE, J.R. (1976). Arterial walls generate from prostaglandin endoperoxides a substance which relaxes strips of mesenteric and coeliac arteries and inhibits platelet aggregation. *Prostaglandins*, **12**, 897–913.

DUSTING, G.J., MONCADA, S. & VANE, J.R. (1977). Prostacyclin (PGX) is the endogenous metabolite responsible for relaxation of coronary arteries induced by arachidonic acid. *Prostaglandins*, **13**, 3–15.

HUGHES, R. (1971). Continuous measurement of peripheral vascular resistance and circulatory function. *Med. biol. Engng.*, **9**, 603–610.

MONCADA, S., GRYGLEWSKI, R., BUNTING, S. & VANE, J.R. (1976). An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature (Lond.)*, **263**, 663–665.