C.31

Decreased plasma protein binding of *o*-methyl red, methyl orange and phenytoin (diphenylhydantoin) in rats with acute renal failure

C.J. BOWMER & W.E. LINDUP

Department of Pharmacology and Therapeutics, University of Liverpool, P.O. Box 147, Liverpool L69 3BX.

Protein binding of drugs is decreased in plasma from uraemic man (Reidenberg, 1977) and animals (Belpaire, Bogaert & Mussche, 1977). To investigate this problem further we have sought a non-surgical animal model for acute renal failure which produces changes in drug binding similar to those observed in man.

Acute renal failure was induced in male and female Wistar albino rats (154–378 g) by intramuscular injection of 50% v/v glycerol (Oken, Cotes, Flamenbaum, Powell-Jackson & Lever, 1975) under light ether anaesthesia. Blood was withdrawn into heparinized tubes by cardiac puncture under ether anaesthesia 24 h post glycerol and the haematocrit, total plasma protein and urea concentrations determined. Equilibrium dialysis at 37°C was used to measure binding (Bowmer & Lindup, 1976). In some experiments binding was measured before and after treatment of the plasma with activated charcoal at pH 3.0 (Craig, Evenson, Sarver & Wagnild, 1976). Dyes were assayed spectrophotometrically and [^{3}H]-phenytoin by liquid scintillation counting. Results are expressed as mean ± s.d.

Glycerol-induced acute renal failure significantly increased plasma urea concentration from 37 ± 10 (n = 67) mg/100 ml (controls) to 299 ± 72 (n = 70) mg/100 ml (uraemic). Total plasma protein concentrations and haematocrit values were not significantly altered in the uraemic rats. Table 1 shows that the binding of phenytoin, *o*-methyl red and methyl orange to uraemic male rat plasma was significantly decreased and similar results (not shown) were obtained with female rats. Charcoal treatment of the uraemic plasma from male rats restored the binding to nearly that of the respective control value (see Table 1).

This model for acute renal failure produces similar decreases in the plasma protein binding of phenytoin, *o*-methyl red and methyl orange to those observed with uraemic human plasma. Furthermore, charcoal treatment substantially restores binding in both rat and human plasma. It appears that charcoal removes one or more inhibitors of drug binding which accumulate in uraemia. Such inhibitors may act directly by occupation of binding sites and/or indirectly by production of a conformational change in the albumin molecule.

C.J.B. was in receipt of an MRC Studentship. This work was partly supported by a grant to W.E.L. from the National Kidney Research Fund.

Table 1 Binding of phenytoin (diphenylhydantoin), *o*-methyl red and methyl orange to uraemic rat plasma before and after extraction with charcoal

	% *Drug bound*†					
	Phenytoin (39.6 μM)		*o-Methyl red* (133 μM)		*Methyl orange* (150 μM)	
	Before	*After*	*Before*	*After*	*Before*	*After*
Control	81.4 ± 0.9	83.9 ± 1.0*	93.3 ± 1.5	93.4 ± 1.1	91.2 ± 0.9	89.0 ± 0.8***
Uraemic	70.7 ± 2.2	81.8 ± 1.8***	74.8 ± 8.0	87.0 ± 2.2**	79.7 ± 4.4	85.5 ± 1.9*

† Each result is the mean (±s.d.) result from 5 male rats.
* $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$ by paired t-test.

References

BELPAIRE, F.M., BOGAERT, M.G. & MUSSCHE, M.M. (1977). Influence of acute renal failure on the protein binding of drugs in animals and in man. *Eur. J. Clin. Pharmac.*, **11**, 27–32.

BOWMER, C.J. & LINDUP, W.E. (1976). Effect of endogenous metabolites on the binding of *o*-methyl red to human serum albumin. *Br. J. Pharmac.*, **58**, 283P–284P.

CRAIG, W.A., EVENSON, M.A., SARVER, K.P. & WAGNILD, J.P. (1976). Correction of protein binding defect in uraemic sera by charcoal treatment. *J. Lab. Clin. Med.*, **87**, 637–647.

OKEN, D.E., COTES, S.C., FLAMENBAUM, W., POWELL-JACKSON, J.D. & LEVER, A.F. (1975). Active and passive immunization to angiotensin in experimental acute renal failure. *Kidney Int.*, **7**, 12–18.

REIDENBERG, M.M. (1977). The binding of drugs to plasma proteins and the interpretation of measurements of plasma concentrations of drugs in patients with poor renal function. *Amer. J. Med.*, **62**, 466–470.