RAT RENAL TUBULE FRAGMENTS: A SENSITIVE PREPARATION FOR THE STUDY OF DRUG INDUCED NEPHROTOXICITY

R. Hernandez Cruz, M. Gumbleton and P.J. Nicholls, Welsh School of Pharmacy, UWIST, PO Box 13, Cardiff CF1 3XF, U.K.

The renal cortical slice has proved to be a useful preparation for assessing drug-induced nephrotoxicity on organic ion transport and metabolic function (Carpenter & Mudge 1980; Kluwe 1981). However, the technique has certain disadvantages, eg. reproducibility of slice thickness and diffusional limitations. These could be overcome by employing an enzyme-dispersed preparation of renal tubule fragments. The present work compares the sensitivity of kidney slices and tubule fragments with reference to the effect of two established nephrotoxins (cephaloridine CEPH and mercuric chloride $HgCl_2$) on 4-aminohippurate (PAH) and inulin uptake and on gluconeogenesis. Kidneys were removed from male Wistar rats (250g) that had received single subcutaneous nephrotoxic doses of either CEPH (100mg/kg) or $HgCl_2$ (2mg/kg), or of normal saline (1ml/kg) 2 days beforehand. Transverse slices of renal cortex 0.5mm thick (average weight 20mg) were rapidly cut (50-70mg/incubation flask) and placed in the incubation medium. Tubule fragments were also prepared from cortical slices by incubation with collagenase and hyaluronidase according to the procedure described by Dawson (1972) and suspended in the medium (2.7-7.5mg cell protein/incubation flask). The medium contained (mM) D-glucose 5.55, Na^+ 140, K^+ 5, Ca^{2+} 2.5, Mg^{2+} 1, Cl^- 144, PO_4^{3-} 3 and SO_4^{2-} 1, buffered to pH 7.4 (Robinson, 1949). Incubation was at 37°C in an atmosphere of 95% O_2/5%CO_2. After an 8 min pre-incubation period, ^{3}H-PAH (to assess organic ion transport) and ^{14}C-insulin (to assess renal extracellular volume), dissolved in the medium, were added to the incubates (final concentrations 50 and 3.3µM respectively; total volume 3ml). After 90 min, radioactivity was determined in renal tissue and medium, and uptake was expressed as T/M (T=dpm/g tissue slice or dpm/g tubule protein; M=dpm/ml incubation medium). To assess gluconeogenesis, pyruvate (10mM) replaced glucose in the medium. Glucose so formed and released into the medium was determined by the glucose oxidase method (Sigma Kit).

Table 1. Effect of two nephrotoxins on rat renal slices and tubule fragments

Pretreatment	PAH uptake (T/M)		Gluconeogenesis (µmol glucose formed/g/h)	
	Slice	Tubule	Slice	Tubule
Saline	3.1 ± 0.2	7.0 ± 0.5	2.2 ± 1.0	327 ± 30
Cephaloridine	3.1 ± 0.3	4.3 ± 0.2*	1.9 ± 1.1	100 ± 90*
$HgCl_2$	2.3 ± 0.2*	4.2 ± 0.2*	1.6 ± 1.4	130 ± 100*

(values are means ± sem. n=5; *P<0.05 compared to saline)

For each of the parameters measured there were differences between the two renal preparations. The uptake of PAH (Table 1) detected the toxicity of CEPH in tubules but not in the slices. $HgCl_2$ significantly ($P<0.05$) decreased the uptake of PAH by both tissue preparations but the magnitude was greater with the tubule fragments. Significant ($P<0.05$) decreases in gluconeogenesis induced by both nephrotoxins were obtained with the tubules but not with the cortical slices. Neither nephrotoxin affected the uptake of inulin in the two preparations (mean T/M for slices 0.27, for tubules 1.8), indicating that the changes determined for tubule fragments represent a more sensitive preparation than renal cortical slices for studies of drug-induced nephrotoxicity in the rat. M.G. is in receipt of a grant from the SERC.

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