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Localization of the nephron site of gentamicin-induced hypercalciuria in the rat: a micropuncture study

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1 *In vivo* renal micropuncture techniques were used to locate the nephron site of hypercalciuria induced by acute gentamicin infusion in anaesthetized Sprague Dawley rats. Three series of experiments were conducted. The effect of gentamicin on calcium reabsorption in the proximal tubule (Series I) and loop of Henle (Series II) was investigated using *in vivo* microperfusion whereas the effect on distal calcium handling (Series III) was studied using *in vivo* microinfusion.

2 In all three experimental series, acute systemic gentamicin infusion at 0.28 mg kg^{-1} min^{-1} caused significant hypercalciuria within 30 min of commencing drug infusion. Gentamicin had no effect on the rates of urine flow or sodium excretion.

3 Acute gentamicin infusion had no effect on unidirectional calcium reabsorption in the proximal tubule or loop of Henle despite a simultaneous and highly significant hypercalciuria at the whole kidney level. Net fluid reabsorption was also unaffected by the drug in these nephron segments.

4 Acute gentamicin infusion significantly increased the urinary recovery of calcium following microinfusion into early distal tubules, whereas urinary calcium recovery was decreased after microinfusion into late distal tubules.

5 We conclude that acute gentamicin-induced hypercalciuria is mediated by a decrease in calcium reabsorption in the early distal tubule. Thus, the acute hypercalciuric effect of gentamicin occurs at a different nephron site to the nephrotoxic effects associated with longer-term administration of the drug. It is, therefore, unlikely that gentamicin-induced hypercalciuria is involved in the pathogenesis of subsequent proximal tubular cell injury.

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Abbreviations: CON, control; GEN, gentamicin; GFR, glomerular filtration rate; MABP, mean arterial blood pressure; NAG, N-acetyl-β-D-glucosaminidase (NAG); PTH, parathyroid hormone

Introduction

The aminoglycoside antibiotics, such as gentamicin, have the potential to cause nephrotoxicity with the principal cellular injury being in the proximal tubule. Nephrotoxicity, often detected as a fall in glomerular filtration rate (GFR), is estimated to occur in 10–20% of patients who receive the drug (Swan, 1997). Gentamicin therapy has also been reported to induce disturbances of electrolyte homeostasis characterized by hypomagnesaemia, hypocalcaemia and hypokalaemia (Watson *et al*., 1983; Zaloga *et al*., 1984; Lanadau & Kher, 1997). These studies have all documented inappropriately high levels of urinary electrolyte excretion for the prevailing low plasma concentrations, indicating a failure of the kidneys to adequately conserve cations.

There is abundant evidence that repeated administration of gentamicin to experimental animals results in increased urinary excretion of divalent cations. Hypercalciuria is the most frequently reported finding (Chahwala & Harpur, 1983; Bennett *et al*., 1985; Cronin & Newman, 1985; Elliott *et al*., 1987; Phipps *et al*., 1990) although hypermagnesiuria has also been reported (Harpur *et al*., 1985).

Acute infusion studies in the anaesthetized rat have revealed that gentamicin treatment decreases the renal tubular reabsorption of calcium and magnesium and that these effects are rapidly reversible on removal of the drug from the infusate (Foster *et al*., 1992; Elliott & Patchin, 1992; Garland *et al*., 1992). Moreover, the effect appears to be specific for divalent cations since sodium and potassium reabsorption were unaffected. Parsons *et al*. (1997) have recently demonstrated for the first time that gentamicin-induced decreases in tubular calcium and magnesium reabsorption can occur (in acute experiments) in the absence of changes in renal tubular morphology as detectable by light and transmission electron microscopy. Although a rapid and dose-related increase in urinary N-acetyl-β-D-glucosaminidase (NAG) excretion was observed in this study, this was attributed to interference with the cellular cycling of NAG rather than a consequence of renal tubular injury. The authors also demonstrated a clear dose-response relationship between the concentration of gentamicin infused and the degree of hypercalciuria and hypermagnesiuria which, consistent with previous studies, was particularly prominent for calcium. Thus, the available evidence suggests that excessive divalent cation excretion represents a pharmacological, rather than a toxicological, response to acute gentamicin infusion.

It is not known exactly where within the nephron that gentamicin has its effect on calcium transport. Such information is important if the mechanism whereby gentamicin induces electrolyte disturbances is to be investigated further. Knowledge of the precise nephron site of action of gentamicin may also indicate whether drug-induced changes in tubular calcium transport are involved in the pathogenesis of

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gentamicin nephrotoxicity. It is known, for example, that the latter process occurs predominantly in the proximal tubule (Kacew, 1990). Clearly if the changes in calcium transport accompanying acute gentamicin infusion occur in the same nephron segment, the two processes might be linked. To date, a single study by Garland *et al.* (1992) using the independent techniques of renal lithium clearance and tubular microinjection of radiolabelled (^{45}Ca) have attempted to grossly locate the nephron site of reduced calcium reabsorption during acute gentamicin infusion. However, firm conclusions concerning the precise nephron site of action of the drug could not be drawn from this study due to the indirect nature of the techniques employed.

The present study aimed to identify the nephron site of gentamicin-induced hypercalciuria using more precise localization techniques than in the previous study. The technique of renal tubular microperfusion was used to investigate the effect of acute gentamicin infusion on unidirectional calcium (^{45}Ca) reabsorption from single proximal tubules and short loops of Henle. Microinfusion, an adaptation of the distal tubular microperfusion technique originally described by Greger *et al.* (1978), was used to study the effect of gentamicin on distal tubular calcium handling. All experiments were performed under conditions which have previously been shown to induce a marked increase in whole kidney urinary calcium excretion (Foster *et al.*, 1992; Garland *et al.*, 1992; Parsons *et al.*, 1997). Preliminary findings of these studies have been published in abstract form (Parsons *et al.*, 1994; 1995).

Methods

Animals

Male Sprague Dawley rats (Charles River Laboratories, Kent, U.K.) weighing 185–320 g were maintained at 21–23°C and exposed to a normal 12 h light cycle (lights on at 0800 h). Animals had free access to food (CRM Labsure; Dorset, U.K.) and water prior to experimental procedures.

Surgical preparation

Rats were anaesthetized with an i.p. injection of sodium thiopentone (Trapanal; Byk-Gulden, Klostanz, Germany) at a dose of 100 mg kg^{-1} and placed on a thermostatically-controlled heated operating table. Body temperature was maintained at 37.0±1.0°C. A tracheostomy was performed to ensure a clear airway. Two polyethylene catheters were inserted in the left jugular vein; one for continuous saline infusion and administration of supplementary anaesthetic when required, and the other for dye injection (see below). A polyethylene catheter was also inserted into the right carotid artery for continuous blood pressure monitoring and collection of blood samples. The left kidney was exposed through a lateral abdominal incision and placed into a perspex cup. The ureter was exposed and a polyethylene catheter inserted for urine collection. A polyethylene catheter was also inserted into the bladder to enable urine collection from the contralateral kidney. The left kidney was immobilized with 3% (w v^{-1}) agar, and its surface continually bathed in liquid paraffin at 37°C.

Experimental protocol

The experimental protocol is outlined in Figure 1. Animals received a continuous infusion of 0.9% (w v^{-1}) NaCl at 200 μl min^{-1} for 2 h after which time the infusion rate was reduced to 100 μl min^{-1} for the remaining 5 h of the experiment. The first 3 h of infusion served for equilibration, the following hour as a control period and the final 3 h as the experimental period. Control animals (CON) were infused with 0.9% (w v^{-1}) NaCl throughout the entire experiment

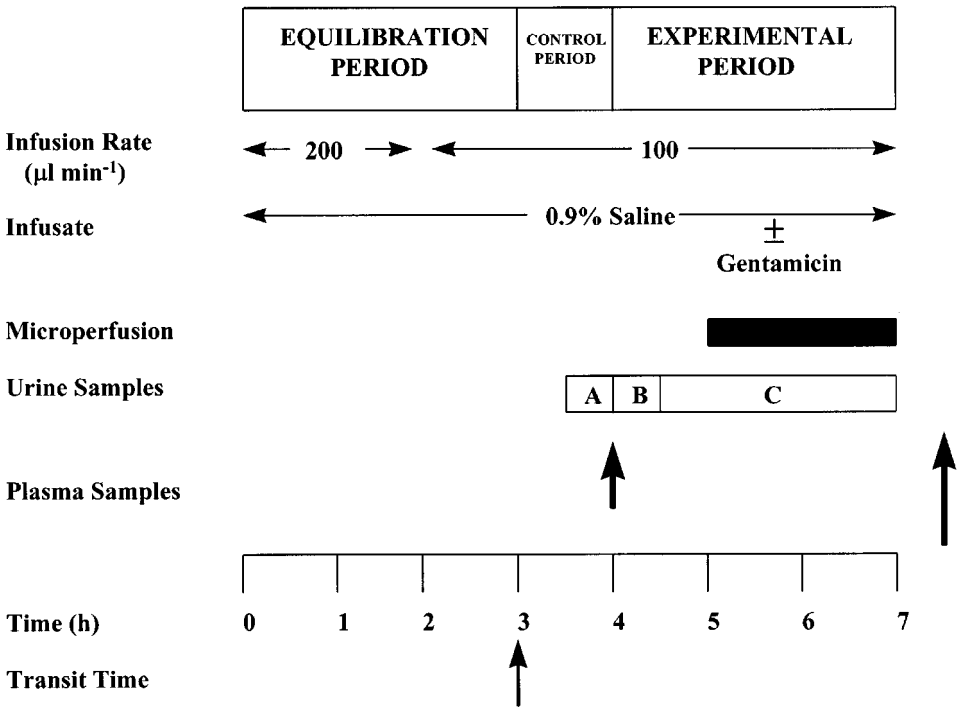

Figure 1 Experimental protocol. There were three urine collection periods (A–C) for proximal and loop microperfusion experiments but only two (A,B) for the distal microinfusion experiments. Micropuncture was performed during the last 2 h of the experimental period. Tubular transit times were assessed and plasma samples obtained at the times indicated by the arrows.

whereas gentamicin infused animals (GEN) received gentamicin base (Cidomycin: Roussel Laboratories Ltd., Uxbridge, U.K.) in 0.9% (w v^{-1}) NaCl during the 3 h experimental period. Three series of experiments were conducted. For the proximal (Series I) and distal (Series III) tubular experiments gentamicin was infused at a rate of 0.28 mg kg^{-1} min^{-1} and for the loop of Henle (Series II) experiments gentamicin was infused at 0.28 or 0.56 mg kg^{-1} min^{-1}. A higher dose of drug was included in second experimental series since previous studies in our laboratory provided some evidence for an effect within the loop of Henle at this higher dose level (Garland *et al*., 1992). The infusion rates used provided a total drug delivery of 50 or 100 mg kg^{-1} respectively over the experimental period. Urine samples were collected into pre-weighed plastic vials over the last 30 min of the control period (sample A), the first 30 min of the experimental period (sample B) and, with the exception of the distal microinfusion experiments, for the remaining 2.5 h of the experimental period (sample C). A small blood sample (150 μl) was taken from the carotid artery at the end of the control period for determination of plasma osmolality. Proximal and loop perfusates (see below) could therefore be adjusted so that they were isosmotic for each animal. A large blood sample (3–4 ml) was collected from the carotid artery on termination of the experiment and used to prepare an ultrafiltrate of plasma using an Amicon Centrifree Micropartition System (Amicon Inc., Beverley, M.A., U.S.A).

The number of animals used in each experimental series was as follows: Series I; CON ($n=12$) and 0.28 mg kg^{-1} min^{-1} GEN ($n=10$), Series II; CON ($n=8$), 0.28 and 0.56 mg kg^{-1} min^{-1} GEN ($n=11$ and 6 respectively), Series III: CON ($n=16$) and 0.28 mg kg^{-1} min^{-1} GEN ($n=12$).

Micropuncture experiments

Renal tubular transit times (Steinhausen, 1963) were measured at the start of the control period by i.v. injection of 5% w v^{-1} erioglaucine (Aldrich, Dorset, U.K.). Only animals with a proximal transit time of <12 s were used. Animals were also excluded if their ureteral transit time exceeded 100 s or if dye retention was observed in the late distal tubule. For all experiments, micropuncture was performed during the last 2 h of the experimental period.

Series I: Proximal tubule For proximal tubule microperfusion randomly selected surface proximal tubules were punctured with a micropipette (5–7 μm tip diameter) and injected with a solution of 0.05% erioglaucine in 0.9% NaCl to determine the course of the nephron and number of further convolutions on the kidney surface. On identification of a proximal tubule with at least three more surface convolutions, a micropipette (7–8 μm) containing bone wax (Ethicon, Edinburgh, U.K.) was inserted into the second surface convolution and a wax block injected into the tubular lumen with the aid of a hydraulic microdrive attached to the pipette (Trent Wells Inc., Coulterville, CA, U.S.A.). The wax was coloured with sudan black (3% w w^{-1}) to aid visibility. The first surface convolution was then repeatedly punctured to allow egress of glomerular filtrate onto the kidney surface and thus prevent any build up of pressure behind the wax block. The third surface convolution (immediately distal to the wax block) was then punctured with the perfusion micropipette (10 μm) and tubular microperfusion commenced at a rate of 25 nl min^{-1} using a perfusion pump (Klaus Effenberger, 8098 Pfaffing/Attel, Germany). The tubule was perfused for 2–3 min prior to inserting a collection micropipette (12 μm) containing castor oil stained with Sudan black (16% w v^{-1}), into the last surface proximal convolution. A column of oil was injected into the tubule and a small negative pressure applied to the pipette to initiate the collection of perfusate. Perfusate was collected over a 3–6 min period. The proximal microperfusion technique is illustrated in Figure 2. On completion of the experiment, perfused segments of proximal tubule were relocated and filled with Microfil (Canton Biomedical Products Inc., Boulder, CO, U.S.A.). The kidney was removed and

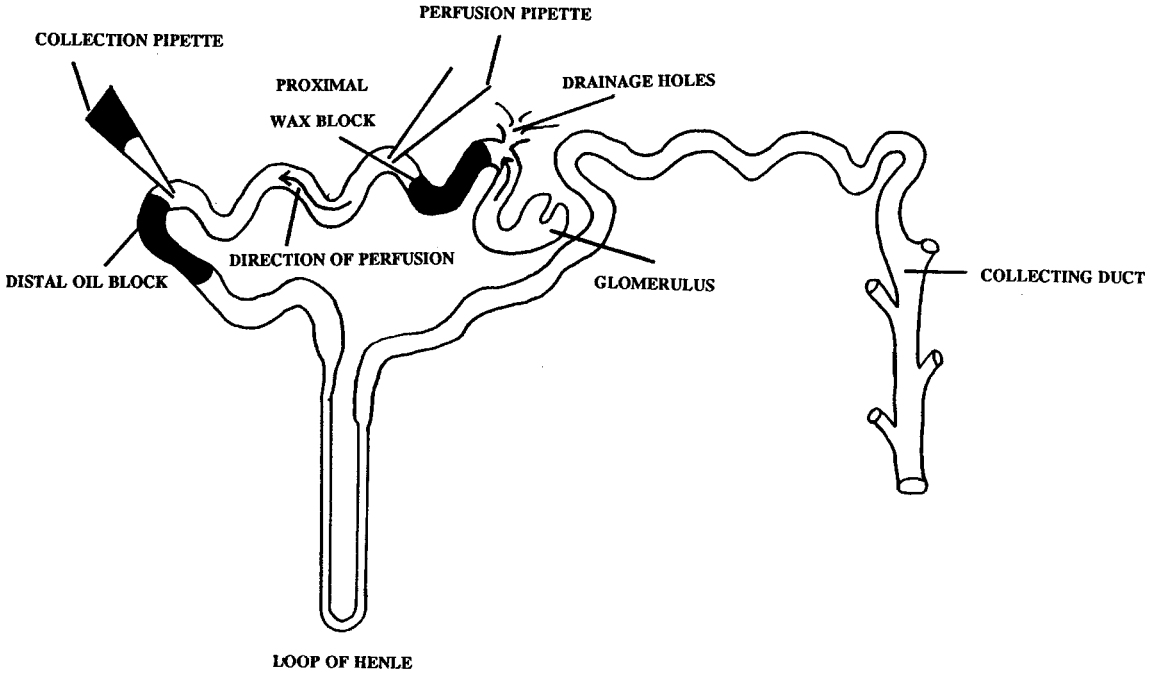

Figure 2 Proximal micoroperfusion technique. Superficial proximal tubules were microperfused at a rate of 25 nl min^{-1} as illustrated for a period of 3–5 min. The tubule was then filled with a silicone rubber compound (Microfil) and a cast of the perfused segment was subsequently dissected from the kidney for determination of the length of tubule perfused.

stored overnight in deionized water at 4°C prior to partial digestion in 20% (v v^{-1}) NaOH and determination of the length of perfused tubule (see below).

Series II: Loop of Henle Microperfusion of short loops of Henle was performed using the same experimental protocol as described above, except that the wax block was placed in the penultimate superficial proximal convolution, the perfusion pipette into the final proximal convolution, and the collection micropipette into the first accessible distal convolution. Loops of Henle were perfused for periods of 3–10 min. It was not possible to fill loops of Henle with Microfil to measure their lengths owing to the small diameter of this nephron segment.

Series III: Distal tubule For distal tubular microinfusion, surface distal tubules were first identified by randomly injecting a solution of 0.05% (w v^{-1}) erioglaucine in 0.9% NaCl into surface proximal convolutions. A wax block was then placed in the last surface proximal convolution and the proximal convolution immediately upstream from this repeatedly punctured to allow an egress of tubular fluid to prevent an increase in pressure behind the block. Prior to microinfusion of the tubule, a timed (5–10 min) urine sample was collected from the ureter of the micropuncture kidney. This served as a blank for the subsequent microinfusion collection. The first available surface distal convolution was then punctured and the tubule perfused for a period of at least twice the ureteral transit time prior to collecting ureteral urine for a similar duration as the blank sample. On completion of microinfusion, the distal tubule was completely filled with Microfil, taking care to maintain the original shape of the nephron. Tubular casts were dissected following partial digestion of the kidney as described earlier, and the location of the microinfusion site along the length of the distal tubule was determined (see below).

The precise composition of perfusates used in each experimental series is given in Table 1. Tritiated inulin [^{3}H]-inulin; Amersham International, plc, Bucks, U.K.) was added to the perfusates to serve as an inert marker allowing tubular fluid reabsorption to be estimated in Series I and II experiments and also to allow perfusate recovery to be determined in all three experimental series. The inclusion of ^{45}Ca (Amersham International, plc, Bucks, U.K.) in the perfusates allowed the determination of unidirectional (lumen to plasma) calcium reabsorption in Series I and II experiments and urinary recovery of calcium following distal tubular microinfusion in Series III experiments. The concentration of gentamicin added to each perfusate was estimated from unpublished data obtained in our laboratory together with textbook values for fluid reabsorption. The former indicated a steady state gentamicin concentration of 26.9 μg ml^{-1} in serum ultrafiltrates obtained from animals infused with gentamicin at 0.28 mg kg^{-1} min^{-1}. For microperfusion of the proximal tubule and loop of Henle, the osmolality of the perfusate was matched to the plasma osmolality of individual animals measured at the end of the control period.

Table 1 Composition of perfusates used in microperfusion and microinfusion experimental series

Constituent	**Series I*	**Series II*	**Series III*
NaCl (mmol l^{-1})	140	150	85
KC1 (mmol l^{-1})	4	4	2
$CaCl_2$ (mmol l^{-1})	1.5	1.5	1
Na_2HPO_4 (mmol l^{-1})	–	2.3	1.5
NaH_2PO_4 (mmol l^{-1})	–	–	3.5
KH_2PO_4 (mmol l^{-1})	–	0.6	–
pH	7.0	6.5	6.5
Erioglaucine (% w v^{-1})	0.1	0.1	0.1
†Gentamicin (μg ml^{-1})	34	67 or 134	300

*All perfusates contained in addition 20.0 μCi ml^{-1} of ^{45}Ca (specific activity, 22.4 mCi mg^{-1}) and [^{3}H]-inulin (specific activity, 0.25 mCi mg^{-1}). †Target gentamicin concentrations; these were subsequently confirmed analytically. For Series II experiments, the concentration of gentamicin added to the perfusate was 67 or 134 μg ml^{-1} for animals infused with gentamicin at a rate of 0.28 or 0.56 mg kg^{-1} min^{-1} respectively.

Analyses

Urine, plasma and plasma ultrafiltrates were analysed for calcium by atomic absorption spectroscopy (Perkin Elmer 3100; Norwalk C.T., U.S.A.). Plasma osmolality was determined by freezing point depression (Roebling Camlab; Cambridge, U.K.) and urinary sodium concentration by flame photometry (Corning EE1 Model 450). The volume of micropuncture samples collected from proximal tubules and loops of Henle was determined by transferring the sample to a constant-bore capillary tube of known diameter. The entire sample was then expelled into liquid scintillant (Optiphase MP; FSA Laboratory Supplies, Leicester, U.K.). The activities of ^{45}Ca and ^{3}H in these samples and urine samples from distal microinfusion experiments were determined by liquid scintillation counting (Packard Tricarb 2000, Canberra Packard, Pangbourne, U.K.) with appropriate corrections made for quenching using the transformed spectral index of the external standard (tSIE) as the quench indicating parameter.

For proximal tubular microperfusions, the length of tubule perfused was determined by measuring the distance between the perfusion and collection sites on a drawing of the tubule cast. Measurement were made with the aid of a microscope plus camera lucida attachment. For distal microinfusions, a similar drawing of the tubule cast was made and the perfusion site, macula densa and site of first convergence with a collecting duct were identified. Distal microinfusions were retrospectively classified as 'early' (perfusion site located within the first 50% of the total distal nephron) or 'late' (perfusion site located within the final 50% of the total distal nephron) tubular microinfusions as described previously (Ellison *et al*., 1987; Velazquez *et al*., 1987).

Calculations and statistics

For proximal and loop microperfusion experiments, net fluid flux (J_v) was calculated as $J_v = V_o.(1-[In_o/In_i])/L$ and unidirectional (lumen to plasma) calcium flux ($J^{lp}Ca$) as $J^{lp}Ca = V_o/L.(Ca_o - Ca_i.[In_o/In_i])$, where V_o is the perfusion rate, In_o is the concentration of inulin in the perfusate, In_i is the concentration of inulin in the collected tubular fluid, Ca_o is the concentration of ^{45}Ca in the perfusate, Ca_i is the concentration of ^{45}Ca in the collected tubular fluid, and L is the length of tubule perfused. For loop microperfusions J_v and $J^{lp}Ca$ were similarly calculated, but were not expressed in terms of tubular length for reasons discussed earlier. For distal microinfusion experiments the fractional urinary recovery of ^{45}Ca ($\%Ca_{rec}$) was calculated as $\%Ca_{rec} = ([Ca_{ur}.100]/[Ca_o.V_o].In_{rec}).100$ where Ca_{ur} is the activity (DPM) of ^{45}Ca in the urine collection and In_{rec} is the percentage recovery of [^{3}H]-inulin.

All data are presented as means ± s.e.mean. All statistical analyses were performed using the Instat Statistical Package (Graphpad Software, California, U.S.A.). For the proximal microperfusion experiments and the distal microinfusion

experiments, differences between the two experimental groups were analysed using Student's *t*-test (two tailed) for unpaired observations. For the loop of Henle microperfusion experiments, a one way analysis of variance (ANOVA) with Scheffe's *post-hoc* procedure was used to investigate significant differences between the three groups. For all experiments, comparisons between control and experimental periods within an experimental series were made using Student's *t*-test for paired observations.

Results

Mean body weights, assessed at the start of each experiment, and mean arterial blood pressure data are presented in Table 2. Body weights were comparable between all groups in all experimental series. Arterial blood pressures were stable throughout the experimental protocol and were unaffected by gentamicin infusion. For all micropuncture studies, the rates of urine flow and sodium excretion were comparable between saline and gentamicin infused animals during both the control and experimental periods. Data for ureteral urine collected from the micropuncture kidney during the initial 30 min of the experimental period are shown in Table 3. Urinary excretion rates for calcium were significantly ($P<0.001$) increased during this period in all animals receiving gentamicin when compared to respective controls. In proximal and loop experiments, significant hypercalciuria was also evident during the remainder of the experimental period (this could not be determined in distal microinfusion studies). Loop experiments showed the magnitude of hypercalciuria to be related to the dose of gentamicin infused. Urinary calcium excretion accompanying gentamicin infusion at 0.56 mg kg^{-1} min^{-1} was significantly ($P<0.01$) higher than that seen with 0.28 mg kg^{-1} min^{-1} gentamicin. Total and ultrafiltrable plasma calcium concentrations were comparable between experimental groups in each micropuncture series (data not presented). Additional general micropuncture data, presented in Table 4, indicate that there were no significant differences between saline and gentamicin infused animals in any experimental series for proximal transit time, late distal transit time or recovery of [^{3}H]-inulin.

Table 2 Mean arterial blood pressure (MABP) and body weight in rats infused with saline (control) or gentamicin (GEN) at 0.28 or 0.56 mg kg^{-1} min^{-1}

Experimental series	*Experimental group*	*MABP* (mmHg)	*Body weight* (g)
Series I	Control ($n=12$)	101 ± 4	234 ± 7
	0.28 GEN ($n=10$)	104 ± 3	225 ± 8
Series II	Control ($n=8$)	100 ± 4	236 ± 5
	0.28 GEN ($n=11$)	104 ± 2	248 ± 5
	0.56 GEN ($n=16$)	98 ± 5	245 ± 8
Series III	Control ($n=16$)	99 ± 4	239 ± 4
	0.28 GEN ($n=12$)	99 ± 3	222 ± 5

Data are presented as means $\pm$ s.e. of the mean, (n) = number of animals. MABP calculated from average data throughout the experiment.

Series I: Proximal tubule In proximal microperfusion studies, the length of tubule (mm) perfused was comparable between saline and gentamicin infused animals (saline = 2.84 ± 0.22 [$n=19$ tubules], GEN = 2.73 ± 0.14 [$n=22$ tubules]). Net fluid reabsorption (nl min^{-1} mm^{-1}) was unaffected by infusion of gentamicin at 0.28 mg kg^{-1} min^{-1} (saline = 1.93 ± 0.17, GEN = 1.77 ± 0.15). The unidirectional (lumen to plasma) reabsorptive flux of ^{45}Ca (pmol min^{-1} mm^{-1}) was also similar in saline and gentamicin infused animals (saline = 7.48 ± 0.33, GEN = 8.13 ± 0.43).

Series II: Loop of Henle Net fluid reabsorption and unidirectional calcium flux in short loops of Henle were comparable between saline and gentamicin infused animals. Moreover, there were no significant differences between the two gentamicin doses for either variable. Values for net fluid reabsorption (nl min^{-1}) were: saline = 7.78 ± 0.35 ($n=17$ tubules), 0.28 GEN = 7.68 ± 0.38 ($n=24$ tubules) and 0.56 GEN = 7.55 ± 0.44 ($n=16$ tubules); and for unidirectional calcium flux (pmol min^{-1}) were: saline = 29.4 ± 1.04, 0.28 GEN = 29.4 ± 0.74 and 0.56 GEN = 28.4 ± 0.72.

Series III: Distal tubule In the distal microinfusion studies, ureteral transit times (seconds) were not significantly affected by gentamicin infusion (saline = 81.5 ± 3.6, GEN = 79.0 ± 3.8). Figure 3 presents data for the urinary recovery of ^{45}Ca from early and late distal tubular microinfusions. The urinary recovery of ^{45}Ca from early distal tubular microinfusions was significantly ($P<0.001$) higher in gentamicin infused animals than in saline infused controls. However, for late distal tubular microinfusions, the urinary recovery of ^{45}Ca was significantly ($P<0.01$) lower in the gentamicin group.

Discussion

The present study confirms the rapid, dose-related hypercalciuria at the whole kidney level which has been previously reported following acute gentamicin infusion in the rat (Foster *et al*., 1992; Elliott & Patchin, 1992; Garland *et al*., 1992; 1994;

Table 3 Urine flow and excretion rates of sodium and calcium in rats infused with saline (control) or gentamicin (GEN) at 0.28 or 0.56 mg kg^{-1} min^{-1}

Experimental series	*Experimental group*	*Flow rate* (μl min^{-1})	*Na excretion* (μmol min^{-1})	*Ca excretion* (μmol min^{-1})
Series I	Control ($n=12$)	32 ± 3	4.9 v 0.3	0.04 ± 0.006
	0.28 GEN ($n=10$)	35 ± 4	5.9 ± 0.5	0.09 ± 0.001***
Series II	Control ($n=8$)	34 ± 4	6.4 ± 0.7	0.04 ± 0.008
	0.28 GEN ($n=11$)	46 ± 3	6.4 ± 0.7	0.13 ± 0.008***
	0.56 GEN ($n=6$)	45 ± 5	7.1 ± 0.5	0.17 ± 0.007***
Series III	Control ($n=16$)	33 ± 3	5.8 ± 0.3	0.04 ± 0.006
	0.28 GEN ($n=12$)	42 ± 2	6.6 ± 0.4	0.11 ± 0.009***

Data are presented as means $\pm$ s.e. of the mean. (n) = number of animals. Data are for urine collected from the ureter of the micropuncture kidney during the initial 30 min of the experimental period (urine collection B, see Figure 1). ***$P<0.001$ comparing GEN with respective control (Students *t*-test or ANOVA with Scheffes *post hoc* test)

Table 4 Tubular transit times and recovery of [^{3}H]-inulin after infusion with saline (control) or gentamicin (GEN) at 0.28 or 0.56 mg kg^{-1}

Experimental series	*Experimental group*	*Proximal transit time* (s)	*Late distal transit time* (s)	*Recovery of [^{3}H]-inulin* (%)
Series I	Control ($n=12$)	7.9±0.5	47.4 v 1.2	97.7±1.5
	0.28 GEN ($n=10$)	7.6±0.5	47.2±0.7	96.5±1.3
Series II	Control ($n=8$)	7.0±0.6	43.4±1.3	99.5±1.5
	0.28 GEN ($n=11$)	7.1±0.4	44.0±1.3	102.9±1.5
	0.56 GEN ($n=6$)	7.5±0.3	44.5±1.2	98.4±1.4
Series III	Control ($n=16$)	7.4±0.3	46.3±0.8	95.5±1.7
	0.28 GEN ($n=12$)	7.3±0.4	44.4±1.0	99.0±2.0

Data are presented as means±s.e. of the mean. (n) = number of tubules perfused for each experimental group.

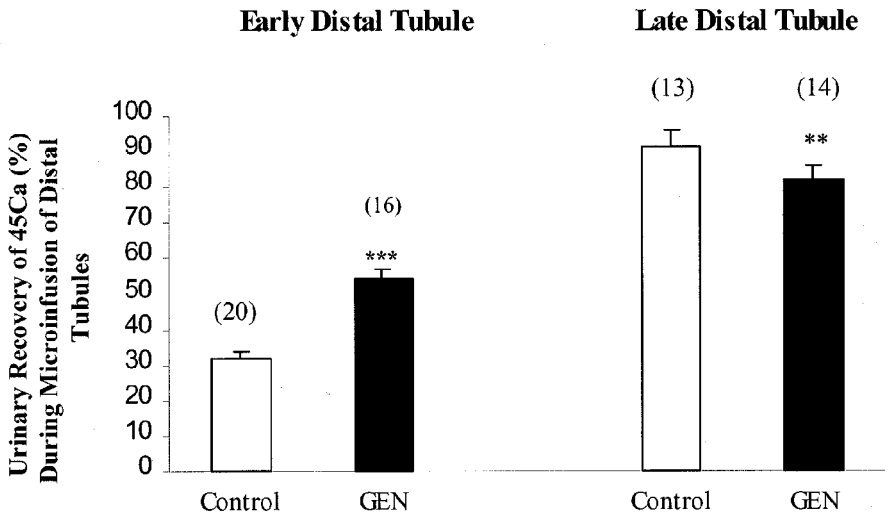

Figure 3 Urinary recovery of ^{45}Ca from micropuncture kidney during microinfusion of early and late distal tubules in animals infused with saline (controls) or gentamicin (GEN) at 0.28 mg kg^{-1} min^{-1}. (n) = number of tubules infused in each experimental group. ***$P=<0.001$; **$P=<0.01$ comparing GEN with respective control group (Student's t-test).

Parsons *et al*., 1997). As with previous studies using the same infusion protocol, the rates of urine flow and sodium excretion were unaffected by acute gentamicin infusion.

Prior to discussing the results of the renal micropuncture experiments, it is appropriate to acknowledge some of the limitations of the techniques used. For the proximal microperfusion experiments, only those segments accessible to micropuncture could be studied thereby excluding the initial 1–2 mm of the proximal convoluted tubule. Thus, an effect on calcium reabsorption specifically at this nephron site would not have been detected. The present study assessed the effect of gentamicin on the unidirectional (i.e. lumen to plasma) reabsorptive calcium flux. It is known that there is a large backflux of calcium from the peritubular plasma into the lumen of the proximal tubule (Murayama *et al*., 1972; Shirley *et al*., 1976). It is possible, therefore, that the hypercalciuria produced by gentamicin could be mediated by an increase in the secretory flux of calcium, which would not have been detected by the present experimental approach. However, Garland *et al*. (1992) showed that acute gentamicin infusion increased the urinary recovery of ^{45}Ca that had been microinjected into the lumen of the proximal tubule. The magnitude of this increase was similar to that observed for non-radioactive calcium at the whole kidney level thus indicating that the drug-induced hypercalciuria probably occurred primarily as a result of a decreased tubular calcium reabsorption.

In order to take account of the possibility that gentamicin exerts its action exclusively at the luminal membrane, in experiments of the nature described here, it is necessary to ensure that the perfusate contains a concentration of gentamicin equivalent to that calculated to be present in the tubular fluid following an intravenous dose of the drug. We have determined the steady-state concentration of gentamicin in ultrafiltrates of serum during gentamicin infusion at 0.28 mg kg^{-1} min^{-1} to be 26.9 μg ml^{-1} (unpublished data). In order to estimate the concentration of gentamicin in tubular fluid delivered to each nephron segment under free-flow conditions, segmental fluid reabsorption and, for the proximal tubule, gentamicin reabsorption, have to be taken into account. It was assumed that 70 and 20% of the filtered fluid load is normally reabsorbed in the proximal tubule and loop of Henle respectively (Koeppen & Stanton, 1992), and that 75% of filtered gentamicin is normally delivered to the late proximal tubule (Senekjian *et al*., 1981). Thus for an infusion rate of 0.28 mg kg^{-1} min^{-1}, tubular fluid gentamicin concentrations should be in the order of 34, 67 and 300 μg ml^{-1} (equivalent to 0.07, 0.14 and 0.62 mM) for the proximal tubule, loop of Henle and distal tubule respectively.

In support of the validity of the experimental model employed in the present study, it is noted that control values for the unidirectional reabsorptive flux of ^{45}Ca and the recovery of ^{45}Ca in final urine, were broadly comparable to those published previously (Murayama *et al*., 1972; Greger *et*

al., 1978; Bishop *et al*., 1981; Garland *et al*., 1991; Johnston & Kau, 1992) when differences in infusion rate are taken into consideration.

The proximal tubule is the principle site of gentamicin-induced renal tubular cell injury (Kacew, 1990) and is quantitatively the most important nephron site for calcium reabsorption (Rouse & Suki, 1990). *In vitro* studies have demonstrated that gentamicin inhibits calcium transport across monolayers of LLC-PK_1 cells of proximal tubular origin (Schwertz *et al*., 1986) and ^{45}Ca uptake into rat proximal tubular cells in primary culture (McGlynn & Ryan, 1993). Taken together, such findings suggest that the proximal tubule is a primary candidate for any investigation into the nephron site of action of gentamicin-induced hypercalciuria. The first attempts to locate the nephron site of gentamicin-induced hypercalciuria were conducted by Garland *et al*. (1992) who, using lithium clearance and ^{45}Ca microinjecton techniques, provided evidence, albeit circumstantial, for a lack of effect of acute gentamicin infusion on proximal calcium transport *in vivo*. The present study demonstrates unequivocally and for the first time, that gentamicin has no effect on the unidirectional (lumen to plasma) reabsorption of ^{45}Ca from the superficial proximal tubule despite a simultaneous and highly significant increase in whole kidney calcium excretion from the micropuncture kidney itself. Harpur *et al*., (1985) originally proposed that early disruption of renal electrolyte handling, particularly calcium, may be a contributory factor in the pathogenesis of subsequent proximal tubular injury rather than a consequence of such injury. In support of this possibility, Parsons *et al*. (1997) demonstrated that the acute effects on renal calcium handling occur independently of and prior to any changes in renal tubular morphology as detectable by light and electron microscopy. However, the findings of the present study dissociate the nephron site of gentamicin-induced hypercalciuria from that of gentamicin nephrotoxicity and indicate that a direct relationship between the two events is unlikely. Information from other studies also supports this contention. Garland *et al*. (1994) for example reported that aminoglycosides with different nephrotoxic potentials induced comparable magnitudes of hypercalciuria. Moreover, prevention of gentamicin-induced renal electrolyte losses by co-administration of amilioride did not protect against subsequent nephrotoxicity (Purnell *et al*., 1985). Finally, acute administration of potassium dichromate, a nephrotoxin specific for the proximal tubule, did not induce hypercalciuria despite evidence of renal injury (Elliott & Patchin, 1992). Thus, the available information indicates the gentamicin-induced hypercalciuria is neither a consequence of, nor is it involved in, the pathogenesis of gentamicin-induced nephrotoxicity.

The loop microperfusion experiments in the present study are the first to show directly that gentamicin has no effect on the reabsorption of calcium in the loop of Henle despite the simultaneous and highly significant whole kidney hypercalciuria. Garland *et al*. (1992) reported that the urinary recovery of ^{45}Ca was significantly increased following microinjection into the proximal tubule although not after microinjection into the distal nephron. Lithium clearance data, used as an indirect marker of proximal calcium reabsorption, provided circumstantial evidence for a lack of effect on proximal calcium handling. Therefore, Garland *et al*. (1992) proposed that gentamicin inhibited calcium reabsorption at a nephron site proximal to the distal nephron and most probably within the loop of Henle. In the present study we were unable to confirm this hypothesis. However, Garland *et al*. (1992) reported that the urinary recovery of ^{45}Ca in saline infused animals was $81.1 \pm 2.0\%$. Greger *et al*. (1978) have shown that the fraction of ^{45}Ca recovered in urine is far higher from late distal tubular microinfusions (about 80% recovery) than from early distal tubular microinfusions (around 15% recovery). The recovery of ^{45}Ca observed in saline infused animals in the present study was 32.0 ± 2.3 and $91.4 \pm 1.5\%$ for early and late distal microinfusions respectively. These data indicate that the distal tubular microinjections performed by Garland *et al*. (1992) were predominantly into the late distal tubule. Therefore, the data presented by Garland *et al*. (1992) are not inconsistent with an effect in the early distal nephron.

In the present experiments, acute gentamicin infusion significantly increased the urinary recovery of ^{45}Ca following microinfusion into early distal tubules with an approximate twofold increase in calcium recovery compared to a simultaneous 3–3.5 fold increase in whole kidney calcium excretion. In the late distal tubule, however there was a significant decrease in the recovery of ^{45}Ca following microinfusion of the drug into this nephron segment. This suggests that the degree of inhibition of ^{45}Ca reabsorption in the early distal tubule may have been larger than that calculated from measurements on the final urine since all distal perfusates will have passed through the distal nephron before being collected and analysed. This may account for the difference between the magnitude of the calciuric effect observed for the whole kidney and at the single nephron level. The observation of a differential effect of gentamicin on calcium reabsorption in early and late distal tubules could be explained by the morphological, physiological and pharmacological heterogeneity of this nephron segment (Brigitte, 1982; Costanzo, 1984; 1985; Kriz & Bankir, 1988).

The present study gives no indication of the mechanism whereby gentamicin inhibits early distal calcium transport. It is unlikely that the drug-induced hypercalciuria occurs simply as a consequence of chelation of intratubular calcium, since this would be expected to occur throughout the nephron and not specifically in the early distal tubule. Furthermore, gentamicin does not chelate calcium in phosphate buffered saline (Kohlepp *et al*., 1982) indicating that this is unlikely to occur *in vivo*. The primary homeostatic regulator of renal calcium excretion is, of course, parathyroid hormone (PTH), which exerts its control at the level of the distal tubule. It is possible therefore, that gentamicin in some way impairs the secretion or action of PTH. In support of this hypothesis, Bennett *et al*. (1985) demonstrated that chronic gentamicin-induced hypercalciuria was attenuated by parathyroidectomy. However, similar acute infusion studies to those employed here have shown the magnitude of gentamicin-induced hypercalciuria to be comparable between parathyroidectomised and parathyroid-intact rats (Foster *et al*., 1988; Elliott & Patchin, 1992).

The mechanisms for basolateral extrusion of calcium from the distal tubule include sodium/calcium exchange and calcium ATPase (Kumar *et al*., 1988; Dominguez *et al*., 1992). There is no direct evidence however that gentamicin can effect either of these systems. The activity of the sodium/calcium exchanger is dependent upon the gradient for sodium maintained by sodium/potassium ATPase activity. Interestingly, gentamicin can inhibit sodium/potassium ATPase in renal tissue (Cronin *et al*., 1982; Chahwala & Harpur, 1983; Fukuda *et al*., 1992; Ali *et al*., 1995) and sodium dependent calcium reabsorption may be decreased under such conditions (Ullrich *et al*., 1976). It is possible, therefore, that gentamicin may inhibit sodium/potassium ATPase and thus reduce the gradient for sodium that drives basolateral sodium/calcium exchange. Given the putative location of the sodium/calcium exchanger, it is feasible that such a mechanism may occur in the distal tubule (Ramachandran & Brunette, 1989). Aminoglycosides have also

been shown to inhibit calcium channels in a number of tissues (Carrado *et al*., 1975; Adams & Durrett, 1978; Mack *et al*., 1992) although such effects have not been investigated in the kidney. Thus, gentamicin-induced hypercalciuria may be mediated by the inhibition of calcium channels which have been demonstrated to be present in the luminal membrane of distal tubular epithelial cells (Bacskai & Friedman, 1990; Friedman & Gesek 1994). Finally, it is interesting to note that the recently discovered renal extracellular calcium-sensing receptor (which occurs in the distal nephron) responds to both calcium and aminoglycoside antibiotics *in vitro* (Riccardi *et al*., 1995; 1996).

There is limited information concerning the potential mechanisms by which gentamicin may enhance calcium reabsorption in the late distal tubule. The uptake of ^{45}Ca into distal luminal membrane vesicles is strongly enhanced by increasing pH (Brunette *et al*., 1992a,b) although it is not known how the vesicles were oriented or which part of the distal tubule they were isolated from. Nevertheless, it is possible that gentamicin may enhance calcium reabsorption in the late distal tubule by decreasing the pH within the lumen of the tubule. For such a mechanism to have occurred in the present study, however, the pH of the perfusion solution would have had to increase *in situ* since the perfusion solution pH was identical for saline and gentamicin infused animals (see Table 1).

In conclusion, this study has used precise localization techniques to demonstrate for the first time that the nephron site where gentamicin acts to reduce tubular calcium reabsorption is the early distal tubule. Although gentamicin caused a small enhancement of calcium reabsorption in the late distal tubule, the net effect on the entire distal nephron was to reduce calcium reabsorption. Gentamicin had no significant effect on calcium reabsorption in the proximal tubule or loop of Henle under conditions where a simultaneous and highly significant hypercalciuria was observed at the whole kidney level. The study demonstrates unequivocally that the acute effects of gentamicin on renal calcium handling occur at a different nephron site to that responsible for the nephrotoxicity associated with longer-term administration of the drug. It is, therefore, unlikely that gentamicin-induced hypercalciuria is involved in the pathogenesis of subsequent proximal tubular cell injury. The cellular mechanism of gentamicin-induced hypercalciuria remains to be elucidated.

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References

ADAMS, R.H. & DURRETT, L.R. (1978). Gentamicin blockade of slow calcium channels in atrial myocardium of guinea pigs. *J. Clin. Invest.,* **78,** 241 – 257.

ALI, B.H., BASHIR, A.A. & TANIRA, M.O.M. (1995). The effect of thyroxine or carbimazole treatment on gentamicin nephrotoxicity in rats. *Human Exp. Toxicol.,* **14,** 13 – 17.

BACSKAI, B.J. & FRIEDMAN, P.A. (1990). Activation of latent calcium channels in renal epithelial cells by parathyroid hormone. *Nature,* **347,** 388 – 391.

BENNETT, W.M., PULLIAM, J.P., PORTER, G.A. & HOUGHTON, D.C. (1985). Modification of experimental gentamicin nephrotoxicity by selective parathyroidectomy. *Am. J. Physiol.,* **249,** F832 – F835.

BISHOP, J.H.V., GREEN, R. & THOMAS, S. (1981). Glucose transport by short loops of Henle in the rat. *J. Physiol,* **320,** 127 – 138.

BRIGITTE, K. (1982). Structural aspects of adaptive changes in renal electrolyte excretion. *Am. J. Physiol.,* **243,** F211 – F226.

BRUNETTE, M.G., MAILLOUX, J. & LAJEUNESSE, D. (1992a). Calcium transport through the luminal membrane of the distal tubule. I. Inter-relationship with sodium. *Kidney Int.,* **41,** 281 – 288.

BRUNETTE, M.G., MAILLOUX, J. & LAJEUNESSE, D. (1992b). Calcium transport through the luminal membrane of the distal tubule. II. Effect of pH, electrical potential and calcium channel inhibitors. *Kidney Int.,* **41,** 289 – 296.

CARRADO, A.P., PRADO, W.A. & DEMORAIS, P. (1975). Competitive antagonism between calcium and aminoglycoside antibiotics in skeletal and smooth muscles. In *Concepts of Membranes in Regulation and Excitation*. ed. Rocha, M., Silva, E. & Suarez-Kurtz, G. pp. 201 – 215. New York: Raven Press.

CHAHWALA, S.B. & HARPUR, E.S. (1983). Gentamicin-induced hypercalciuria in the rat. *Acta Pharmacol. Toxicol.,* **53,** 358 – 362.

COSTANZO, L.S. (1984). Comparison of calcium and sodium transport in early and late rat distal tubules: effect of amiloride. *Am. J. Physiol.,* **246,** F937 – F945.

COSTANZO, L.S. (1985). Localisation of diuretic action in microperfused rat distal tubules: sodium and calcium transport. *Am. J. Physiol,* **248,** F527 – F535.

CRONIN, R.E. & NEWMAN, J.A. (1985). Protective effect of thyroxine but not parathyroidectomy on gentamicin nephrotoxicity. *Am. J. Physiol.,* **248,** F332 – F339.

CRONIN, R.E., NIX, K.L., FERGUSON, E.R., SOUTHERN, P.M. & HENRICH, W.L. (1982). Renal cortex ion composition and Na/K ATPase activity in gentamicin nephrotoxicity. *Am. J. Physiol.,* **242,** F477 – F483.

DOMINGUEZ, J.H., JUHASHZOUI, M., KLEIBOEKER, S.B., HALE, C.C. & FEISTER, H.A. (1992). Sodium-calcium exchanger of rat proximal tubule: gene expression and subcellular localisation. *Am. J. Physiol.,* **263,** F945 – F950.

ELLIOTT, W.C. & PATCHIN, D.S. (1992). Aminoglycoside-mediated calciuresis. *J. Pharmacol. Expt. Ther.,* **262,** 151 – 156.

ELLIOTT, W.C., PATCHIN, D.S. & JONES, D.B. (1987). Effect of parathyroid hormone activity on gentamicin nephrotoxicity. *J. Lab. Clin. Med.,* **109,** 48 – 54.

ELLISON, D.H., VELAZQUEZ, H. & WRIGHT, F.S. (1987). Thiazide-sensitive sodium transport in early distal tubule. *Am. J. Physiol.,* **253,** F546 – F554.

FOSTER, J.E., HARPUR, E.S. & GARLAND, H.O. (1992). An investigation of the acute effect of gentamicin on the renal handling of electrolytes in the rat. *J. Pharmacol. Exp. Ther.,* **261,** 38 – 43.

FOSTER, J.E., HARPUR, E.S. & PERRIS, A.D. (1988). Evidence that the hypercalciuric and hypermagnesiuric responses to gentamicin in the rat are not mediated by inhibition of parathyroid hormone. *J. Physiol.,* **403,** 19 p.

FRIEDMAN, P.A. & GESEK, F.A. (1994). Hormone-responsive calcium entry in distal convoluted tubules. *J. Am. Soc. Nephrol.,* **4,** 1396 – 1404.

FUKUDA, Y., EKLOF, A.C., MALMBORG, A.S. & APERIA, A. (1992). Calcium supplementation and thyroid hormone protect against gentamicin-induced inhibition of proximal tubular Na/K ATPase activity and other renal functional changes. *Acta Physiol. Scand.,* **145,** 93 – 98.

GARLAND, H.O., BIRDSEY, T.J., DAVIDGE, C.G., McLAUGHLIN, L.M., OAKES, A.J., SMITH, A.J. & HARPUR, E.S. (1994). Effects of gentamicin, neomycin and tobramycin on renal calcium and magnesium handling in two rat strains. *Clin. Expt. Pharmacol. Physiol.,* **21,** 109 – 115.

GARLAND, H.O., HARRIS, P.J. & MORGAN, T.O. (1991). Calcium transport in the proximal convoluted tubule and loop of Henle of rats made diabetic with streptozotocin. *J. Endocriniol.,* **131,** 373 – 380.

GARLAND, H.O., PHIPPS, D.J. & HARPUR, E.S. (1992). Gentamicin-induced hypercalciuria in the rat: assessment of nephron site involved. *J. Pharmacol. Expt. Ther.,* **263,** 293–297.

GREGER, R., LANG, F. & OBERLEITHNER, H. (1978). Distal site of calcium reabsorption in the rat nephron. *Pflügers Arch.,* **374,** 153–157.

HARPUR, E.S., FATHALLA, S.A.K. & PHIPPS, D.J. (1985). Increased urinary excretion of calcium and magnesium is an early manifestation of gentamicin-induced nephrotoxicity in the rat. In *Renal Heterogeneity and Target Cell Toxicity*. ed. Bach, P.H. pp. 349–352. Chichester: John Wiley & Sons.

JOHNSTON, P.A. & KAU, S.T. (1992). Evaluation of the renal site of action of a novel, eukalaemic diuretic, ICI 207828. *J. Pharmacol. Expt. Ther.,* **260,** 458–462.

KACEW, S. (1990). Pathogenic factors in aminoglycoside-induced nephrotoxicity. *Toxicol. Lett.,* **51,** 241–259.

KOEPPEN, B.M. & STANTON, B.A. (1992). Renal fluid and electrolyte reabsorption. In *Renal Physiology*. ed. Koeppen, B.M. & Stanton, B.A. pp. 49–69. Missouri, USA: Mosby Year Book.

KOHLEPP, S.J., PLANT, S.B., MCCARRON, D.A. & GILBERT, D.N. (1982). Gentamicin does not chelate calcium. *Antimicrob. Agents Chemother.,* **21,** 668–669.

KRIZ, W. & BANKIR, L. (1988). A standard nomenclature for structures of the kidney. *Am. J. Physiol.,* **254,** F1–F8.

KUMAR, R., BORKE, J.L., CARIDE, A., VERMA, A.K. & PENNISTON, J.T. (1988). Localisation of erythrocyte Ca/Mg ATPase and 28-KDa-calcium binding protein in the rat kidney distal tubules. *Clin. Res.,* **36,** 595A.

LANADAU, D. & KHER, K.K. (1997). Gentamicin-induced Bartter-like syndrome. *Paed. Nephrol.,* **11,** 737–740.

MACK, M.M., ZIMANYI, I. & PESSAH, I.N. (1992). Discrimination of multiple binding sites for antagonists of the calcium release channel complex of skeletal and cardiac sarcoplasmic reticulum. *J. Pharmacol. Exp. Ther.,* **262,** 1028–1037.

MCGLYNN, H. & RYAN, M.P. (1993). *In vitro* mechanisms and models of gentamicin nephrotoxicity. *Toxicol. Lett.,* **53,** 197–200.

MURAYAMA, Y., MOREL, F. & LEGRIMELLEC, C. (1972). Phosphate, calcium and magnesium transfer in proximal tubules and loops of Henle as measured by single nephron microperfusion experiments in the rat. *Pflügers Arch.,* **333,** 1–16.

PARSONS, P.P., GARLAND, H.O. & HARPUR, E.S. (1994). Assessment of the dose-response relationship and nephron site of gentamicin-induced hypercalciuria in the anaesthetised rat. *Human Exp. Toxicol.,* **13,** 284.

PARSONS, P.P., GARLAND, H.O. & HARPUR, E.S. (1995). Effect of gentamicin on urinary recoveries of 45Ca from distal tubular microperfusions in the anaesthetised rat. *J. Physiol.,* **483,** 168P.

PARSONS, P.P., GARLAND, H.O., HARPUR, E.S. & OLD, S. (1997). Acute gentamicin-induced hypercalciuria and hypermagnesiuria in the rat: dose-response relationship and role of renal tubular injury. *Br. J. Pharmacol.,* **122,** 570–576.

PHIPPS, D.J., SPENCER, A.J. & HARPUR, E.S. (1990). Comparison of the renal response of male Fischer 344, Sprague Dawley and Wistar rats to administration of gentamicin. *Toxicol. Lett.,* **53,** 203–205.

PURNELL, J., HOUGHTON, D.C., PORTER, G.A. & BENNETT, W.M. (1985). Effect of amiloride on experimental gentamicin nephrotoxicity. *Nephron,* **40,** 166–170.

RAMACHANDRAN, C. & BRUNETTE, M.G. (1989). The renal Na/Ca exchange system is located exclusively in the distal tubule. *Biochem. J.,* **257,** 259–264.

RICCARDI, D., LEE, W.-S., LEE, K., SEGRE, G.V., BROWN, E.M. & HERBERT, S. (1996). Localization of the extracellular calcium-sensing receptor and PTH/PTHrP receptor in rat kidney. *Am. J. Physiol.,* **271,** F951–956.

RICCARDI, D., PARK, J., LEE, W.-S., GAMBA, G., BROWN, E.M. & HERBERT, S. (1995). Cloning and functional expression of a rat kidney extracellular calcium/polyvalent cation-sensing receptor. *Proc. Natl. Acd. Sci., USA,* **92,** 131–135.

ROUSE, D. & SUKI, W.N. (1990). Renal control of extracellular calcium. *Kidney Int.,* **38,** 700–708.

SCHWERTZ, D.W., KREISBERG, J.I. & VENKATACHALAM, M.A. (1986). Gentamicin-induced alterations in pig kidney epithelial ($LLC\text{-}PK_1$) cells in culture. *J. Pharmacol. Exp. Ther.,* **236,** 254–262.

SENEKJIAN, H.O., KNIGHT, T.F. & WEINMAN, E.J. (1981). Micropuncture study of the handling of gentamicin by the rat kidney. *Kidney Int.,* **19,** 416–423.

SHIRLEY, D.G., POUJEOL, P. & LEGRIMELLEC, C. (1976). Phosphate, calcium and magnesium fluxes into the lumen of the rat proximal convoluted tubule. *Pflügers Arch.,* **362,** 247–254.

STEINHAUSEN, M. (1963). Eine methode zur differenzierung proximaler und distaler tubuli der nierenrinde von ratten *in vivo* und ihre anwendung zur bestimmung tubularer stromungsgeschwindigkeiten. *Pflügers Arch.,* **277,** 23–35.

SWAN, S.K. (1997). Aminoglycoside nephrotoxicity. *Sem. in Nephrol.,* **17,** 27–33.

ULLRICH, K.J., RUMRICH, G. & KLOSS, C.R. (1976). Active Ca reabsorption in the proximal tubule of the rat kidney. *Pflügers Arch.,* **364,** 223–228.

VELAZQUEZ, H., ELLISON, D.H. & WRIGHT, F.S. (1987). Chloride-dependent potassium secretion in early and late renal distal tubules. *Am. J. Physiol.,* **253,** F555–F562.

WATSON, A., HOLOHAN, P.D. & ROSS, C.R. (1983). Severe hypomagnesaemia and hypocalcaemia following gentamicin therapy. *Ir. Med. J.,* **76,** 381–383.

ZALOGA, G.P., CHERNOW, B., POCK, A., WOOD, B. & ZARITSKY, A. (1984). Hypomagnesaemia is a common complication of aminoglycoside therapy. *Surg. Gynaecol. Obstet.,* **158,** 561–565.

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