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J. MANITIUS**, E. J. JOHNS, A. L. CHAMIENIA*

THE EFFECT OF NITRENDIPINE ON RENAL HAEMODYNAMICS AND TUBULAR REABSORPTION AND ITS NEURAL CONTROL IN ANAESTHETISED RATS WITH CHRONIC RENAL FAILURE

Department of Physiology, The Medical School, University of Birmingham, United Kingdom, * Department of Nephrology, Medical University, Gdansk, Poland, ** Department of Nephrology, Medical University Bydgoszcz, Poland

This study examined the influence of a calcium channel antagonist, nitrendipine, on blood pressure and kidney function in a rat model of chronic renal failure. Additionally, the effects of low frequency renal nerve stimulation were studied in the presence and absence of nitrendipine. Male Wistar rats were fed a diet high in adenine for 4 weeks and then acutely anaesthetised and prepared for renal functional measurements. Blood pressure was elevated but renal blood flow and glomerular filtration rate were reduced, between 30 to 50%, urine flow and absolute sodium excretion were lower and fractional sodium excretion was two to three times higher than in normal rats. Nitrendipine (0.25μg/kg/min i.v.) decreased blood pressure at 114 ± 7 mmHg, by 11% ($P<0.05$), increased left renal blood flow, at 1.3 ± 0.2 ml/min $^{1}g^{1}$, by 16% ($P<0.01$), and urine flow, absolute and fractional sodium excretions, by between 50—83% (all $P<0.05$). Renal nerves stimulation (0.7—1.3 Hz, 15V, 0.2 ms) decreased ($P<0.02$) left renal blood flow by 10% but had no effect on excretory variables, irrespective of nitrendipine administration. These results show that in renal failure rats the vascular and tubular responses to nitrendipine are preserved. However, the neural regulation of tubular reabsorption is abolished in this experimental model, irrespective of nitrendipine administration.

Key words: *calcium channel antagonists, chronic renal failure, renal nerves*

INTRODUCTION

The calcium channel antagonists have become an established class of compounds for the treatment of hypertensive and cardiac diseases (1, 2). They operate by blocking calcium channels, primarily of the L-type, thereby preventing the inward movement of calcium, primarily to smooth muscle cells. At the level of the heart, there is a delayed transmission along the conducting system and there is decreased contractility which protects the ventricular

muscle cells from over excitability. Furthermore, at vascular smooth muscles, inhibition of calcium entry into the cells results in hyperpolarisation, a dilatation of the muscle cells, a decrease in peripheral resistance and hence a fall in blood pressure (3). The influence of calcium channel antagonists on the kidney is complex because of the close interaction between vascular and epithelial tissue function. Early reports from this laboratory (4, 5) demonstrated that administration of diltiazem and nifedipine at doses which had only small effects on blood pressure and renal haemodynamics caused a natriuresis and diuresis but whether this was a direct action on the tubular reabsorptive processes or some change in intra-renal haemodynamics has not been resolved. An action at the tubular level seems unlikely as a micropuncture study by Haberle *et al.* (6) was unable to show any effect of the calcium channel antagonists at the early distal tubule while studies from this laboratory with isolated proximal tubules failed to show any effect on sodium handling (7). However, there is evidence that these drugs cause arteriolar dilatation and blunt the vasoconstriction of angiotensin II preferentially at the pre- rather than post-glomerular resistance bed (8). This received support from the observations that following administration of calcium channel antagonists, autoregulation of blood flow through the kidney is abolished (9), an action which appears to occur primarily at the cortical vasculature (10, 11). Moreover, it is likely that diminished vascular responses to renal perfusion pressure changes induced by the calcium channel antagonists cause the blunting of the pressure-natriuresis relationship (12).

At the present time, the usefulness and effectiveness of the calcium channel antagonists in the treatment of hypertension associated with chronic renal failure, is being actively debated (13, 14). Therefore this investigation set out to examine the effects of acute administration of a calcium channel antagonist on renal haemodynamic and excretory function in a rat model of chronic renal failure induced by adenine feeding (15). This was extended to determine whether the neural regulation of fluid handling by the kidney was affected in any way by the calcium channel antagonist. This was done by assessing the effects of renal denervation and low frequency renal nerve stimulation on renal haemodynamics and excretory function in the absence and presence of a calcium channel antagonist nitrendipine.

METHODS

Experiments were undertaken in male Wistar rats (mean body weight 280—340 g) which had been fasted overnight. All animals were fed a standard rat diet which contained adenine (0.75% w/w) for 4—5 weeks prior to the clearance experiments (16). Animals were anaesthetised with 60 mg kg^{-1} body weight i.p. and maintained with a constant infusion of pentobarbitone sodium (12 mg kg^{-1} h^{-1}). After anaesthesia was achieved, a tracheostomy was performed and animals breathed spontaneously throughout the experiment. Polyethylene catheters were placed in the femoral artery

to allow blood pressure measurements and blood sampling and in the femoral vein for experimental infusions. An intravenous infusion of isotonic saline (3 ml h^{-1}) was commenced immediately and continued for the duration of the experiment. The right ureter was cannulated *via* a flank incision. The left kidney was exposed by a retroperitoneal approach, its ureter cannulated and artery carefully cleared and an electromagnetic flow probe (2.0—2.5 mm internal circumference, EP100 series, Carolina Medical Instruments) was placed around the artery. The left renal nerves were identified under a surgical microscope, carefully dissected and cut. The arterial catheter was connected to a pressure transducer (Statham P23 I) and the signal fed to a custom built amplifier (Grayden, Birmingham). The flow meter probe was connected to a square wave electromagnetic flow meter (Model FM501, Carolina Medical Instruments, U.S.A.). Blood pressure and renal blood flow signals were then fed *via* an I/O card to an Apple Macintosh computer running custom software written in LabView (National Instruments, Austin, TX, U.S.A.) and displayed on the screen. Mean values for all variables were calculated for every 2 s and then averaged over each of 15 min clearance periods. Data was stored on the hard disk for later off-line analysis. On completion of the surgery isotonic saline was replaced by inulin in saline (1.5 g 100 ml^{-1}) and was infused at 3 ml h^{-1} and the animals were allowed to recover for two hours before the clearance experiments were started.

Experimental protocols:

The experimental protocol consisted of seven clearance periods, 15 min each. Two control clearances were performed, followed 20 minutes later by a series of another five clearances (two basal, one experimental and two recovery clearances). Before the experimental period started, the distal portion of the left renal nerve was placed on a bipolar stimulating electrode. The nerve was then stimulated (15 V, 0.2 ms) throughout the experimental clearance at low frequency (0.7—1.3 Hz) adjusted to cause an approximate 10% fall in the left kidney blood flow (RBF). Arterial blood samples (300 µl each) were taken before the first and then after the end of the second, fourth, fifth and seventh clearance periods. The blood samples were immediately centrifuged and plasma obtained, the red cells were resuspended in an equivalent volume of heparinized saline and reinfused into the animal within 5 min. Urine was collected in preweighed microcentrifuge capped tubes. Two groups of animals were studied:

Group I: (n = 6) served as controls. After two baseline clearance periods were completed, vehicle was added to the infusion of inulin in saline at the same concentration as in animals receiving nitrendipine and this was continued until the end of the experiment.

Group II: (n = 7). After two baseline clearance periods were completed, nitrendipine (1 mg ml^{-1} in a 969 : 60 : 100 polyethyleneglycol 400 : water mixture) was added into the inulin in saline infusion in such concentration as to achieve a delivery rate of 0.25 µg kg^{-1} min^{-1} and continued until the end of the experiment.

Chemical assays:

Urinary and plasma electrolyte concentrations were measured by flame photometry (Ciba Corning 410C). Plasma and urine samples inulin was measured as described previously (17).

Statistical analysis:

All values are presented as means ± S.E.M. The mean values of two baseline, two basal and two recovery values were calculated. Statistical analysis was performed with repeated measures analysis of variance (SuperANOVA software, Abbacus Concept, Berkeley, CA, U.S.A.) and changes were taken to be significant when $P < 0.05$.

RESULTS

The glomerular filtration rates (*Table 1*) observed in adenine fed rats were substantially lower than those observed by us in normal animals in earlier experiments (19) but comparable to those reported previously in this model of renal failure (16). In both groups the glomerular filtration rate, urine flow and absolute sodium excretion were significantly higher in denervated than in innervated kidneys (Group I: all $P < 0.001$; Group II: all $P < 0.001$) whereas fractional sodium excretions were not different between denervated and innervated kidneys in either group.

Table 1. The effect of vehicle or nitrendipine on haemodynamics and renal water and sodium excretion in chronic renal failure rats.

	Vehicle group (n = 6)		Nitrendipine group (n = 7)	
	Before	After	Before	After
Blood pressure, mmHg	122±6	116±8	114±7	10±6***
DENERVATED KIDNEY:				
Renal blood flow, ml $min^{-1}g^{-1}$	1.6±0.2	1.5±0.2	1.3±0.2	1.5±0.2**
Glomerular filtration rate, ml $min^{-1}g^{-1}$	0.20±0.07	0.20±0.03	0.17±0.04	0.19±0.02
Urine flow rat, µl $min^{-1}g^{-1}$	10.0±0.9	10.7±1.6	7.4±0.8	10.2±0.8*
Absolute sodium excretion, µmol $min^{-1}g^{-1}$	0.82±0.18	1.02±0.34	0.60±0.11	1.05±0.22*
Fractional sodium excretion, %	3.48±0.9	3.66±0.98	2.57±0.47	4.00±0.70**
INNERVATED KIDNEY:				
Glomerular filtration rate, ml $min^{-1}g^{-1}$	0.13±0.03	0.11±0.02	0.10±0.02	0.11±0.02
Urine flow rate, µl $min^{-1}g^{-1}$	5.8±0.5	5.8±0.6	4.6±0.8	7.4±2.0*
Absolute sodium excretion, µmol $min^{-1}g^{-1}$	0.41±0.08	0.43±0.12	0.30±0.07	0.70±0.29*
Fractional sodium excretion, %	2.69±0.52	2.87±0.51	2.49±0.69	5.28±2.16*

* — significantly different from respective values obtained before either vehicle or nitrendipine, $P < 0.05$, ** $P < 0.001$, *** $P < 0.001$.

The effect of either vehicle or nitrendipine infusion on renal haemodynamics and excretory function is shown in *Table 1*. There were no significant changes in any of the variables resulting from the vehicle infusion which remained stable for the duration of the experiment. Nitrendipine infusion in Group II animals resulted in a significant reduction of arterial pressure, by 11% ($P < 0.001$), and increased renal blood flow, by some 16% ($P < 0.01$). At the same time urine flow, absolute and fractional sodium excretion all increased significantly in both innervated and denervated kidneys (UV by 50 and 63%, $P < 0.05$; $U_{Na}V$ by 81 and 110%, $P < 0.05$; FE_{Na} by 60 and 81%, $P < 0.05$). These increases in water and sodium excretions were accompanied by much smaller increases in GFR of 12% and 17% in denervated and innervated kidney respectively which did not reach statistical significance.

Renal nerve stimulation at low frequencies (0.7—1.3 Hz) caused a small, albeit significant, fall in left kidney renal blood flow in both the vehicle group and nitrendipine treated animals (of $-10.5 \pm 3.0\%$, $P < 0.01$ and $-10.3 \pm 1.5\%$ $P < 0.02$, respectively) while mean arterial pressure did not change (*Fig. 1*).

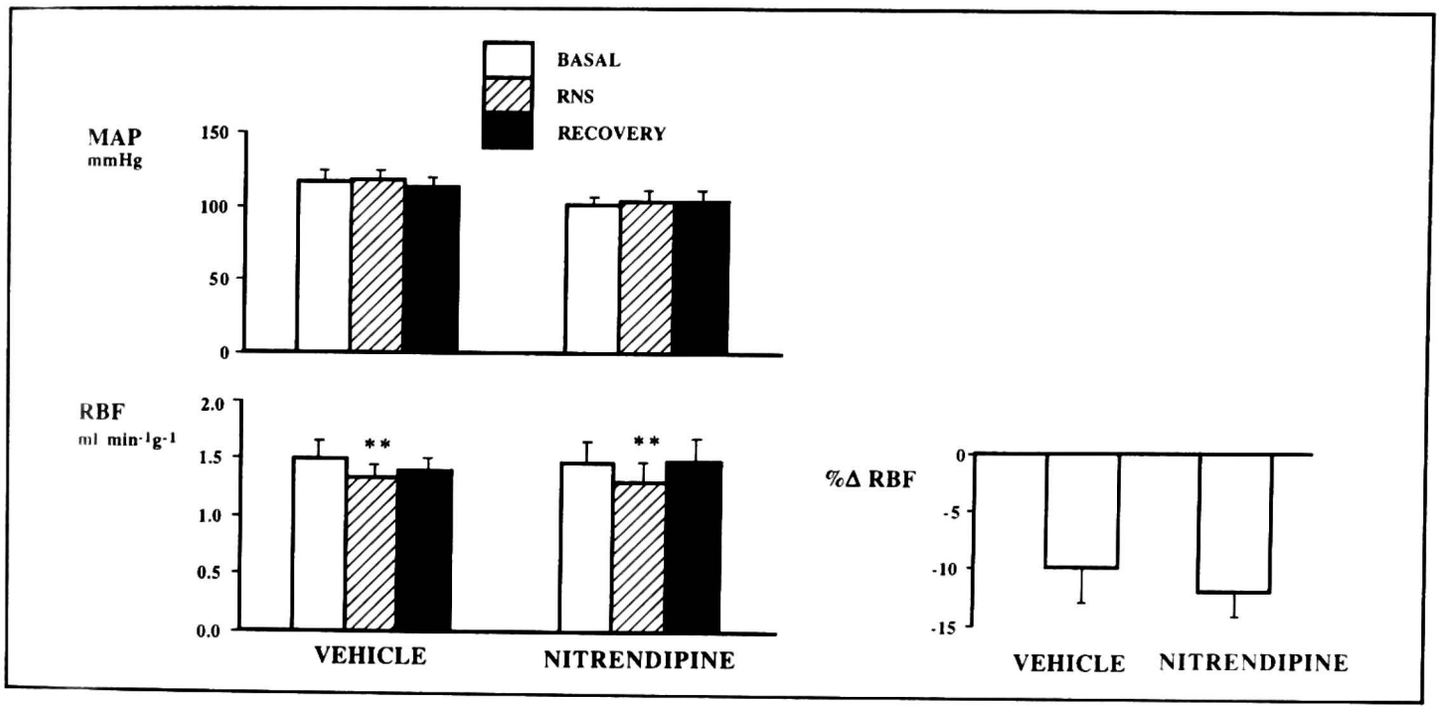

Fig. 1. This shows the absolute values of mean arterial pressure (MAP) and renal blood flow (RBF) before, during and after low frequency renal nerve stimulation (RNS) in chronic renal failure rats in the absence and presence of nitrendipine. The right hand panel shows the percentage change in renal blood flow caused by the renal nerve stimulation. ** = $P < 0.01$ comparison between the RNS period and the average of the basal and recovery periods.

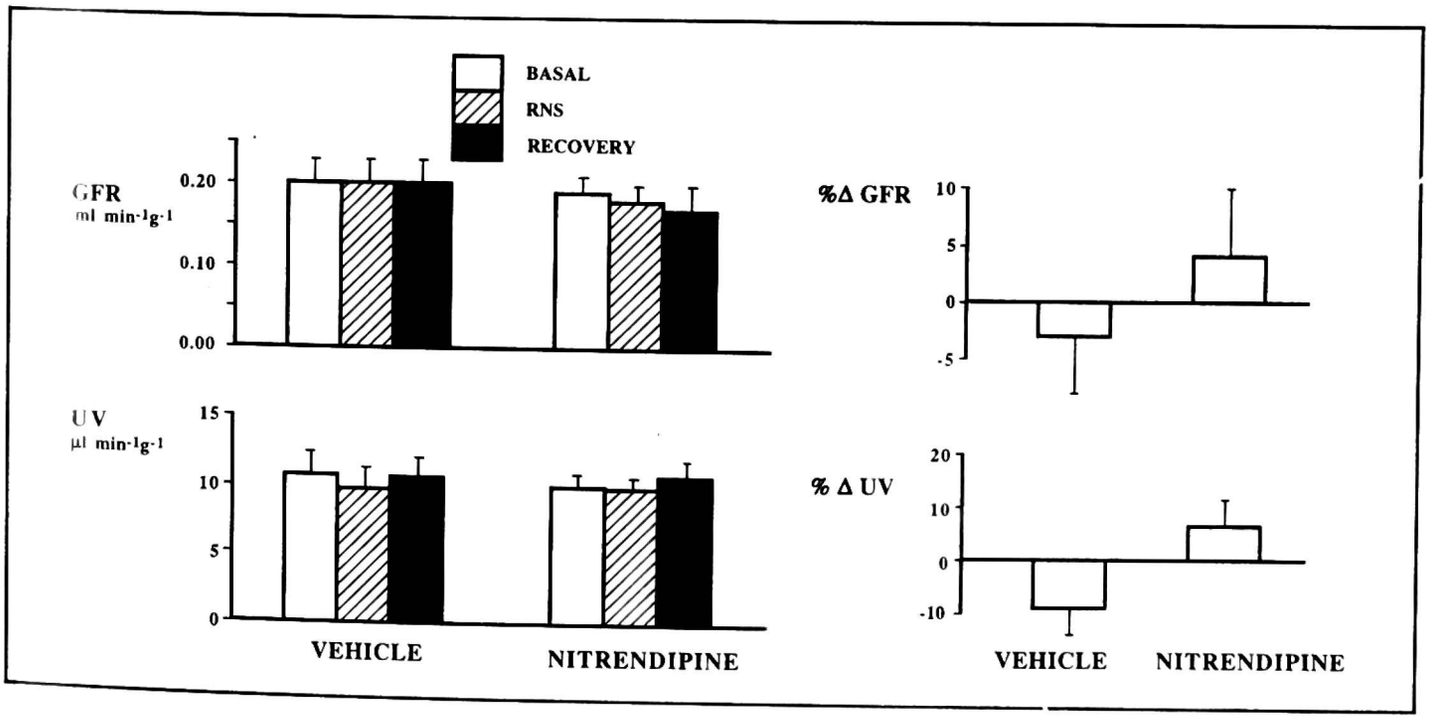

Fig. 2. This shows the absolute values of glomerular filtration rate (GFR) and urine flow (U_V) before (basal), during and after (recovery) low frequency renal nerve stimulation (RNS) in chronic renal failure rats receiving either vehicle or nitrendipine (0.25 $\mu g kg^{-1} min^{-1}$). The right hand panel illustrates the percentage changes caused by the RNS.

Under these conditions no changes in GFR, UV (*Fig. 2*), or $U_{Na}V$ or FE_{Na} (*Fig. 3*) were observed in either the vehicle infused or the animals given nitrendipine.

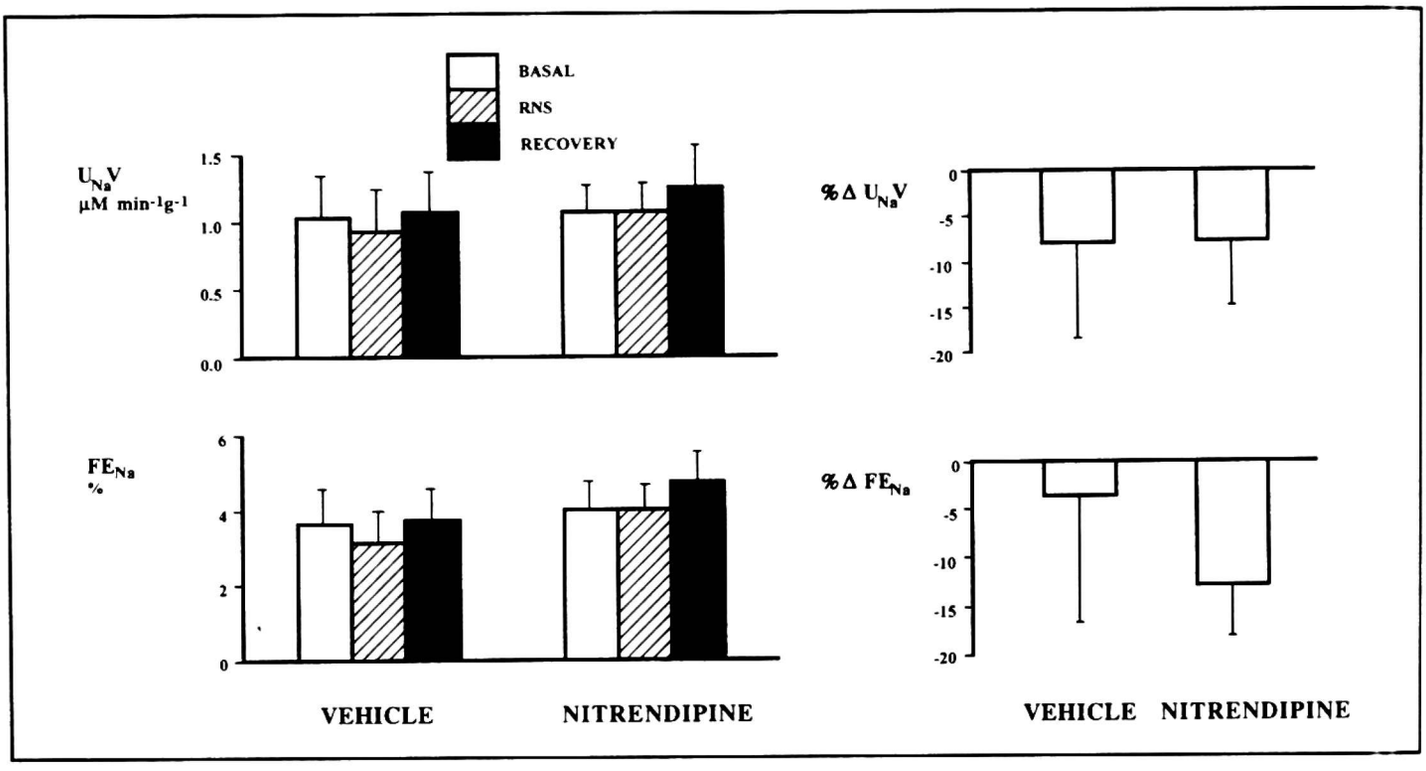

Fig. 3. This presents the absolute ($U_{Na}V$) and fractional (FE_{Na}) sodium excretions before (basal), during and after (recovery) low frequency renal nerve stimulation (RNS) in chronic renal failure rats receiving either vehicle or nitrendipine (0.25 μgkg ^{1}min ^{1}). The right hand panel demonstrates the percentage changes caused by the RNS.

DISCUSSION

The main thrust of this study was to examine the impact of the calcium channel antagonist on the haemodynamic and tubular function in kidneys whose activity had been compromised and could be considered to be in a state of chronic renal failure. The approach taken was to determine the effect of administering the calcium channel antagonist, nitrendipine, on basal function and on the neural control of kidney function.

The model of chronic renal failure which was chosen was based on that described by Yokazawa *et al.* (15) who utilised a period of adenine feeding. Under these conditions, there was precipitation of the compound along the tubules as fluid was concentrated with the result that there was a gradual loss of nephron function as occurs progressively in chronic renal failure in man. This group (15) found that after 30 to 40 days of adenine feeding, serum creatinine and urea levels were raised by 3.4 and 7 fold, respectively, indicative of a marked reduction in haemodynamic function. This was supported by their histological findings that whilst the tubules had crystalline deposits and interstitial fibrosis had developed, the glomeruli were relatively normal.

Moreover, serium calcium was reduced, by about 25% and phosphate was correspondingly raised. It was against this background that the present study was performed.

In a previous report from this laboratory using this model (16) in which acute measurements of renal haemodynamic and excretory function were performed, it was found that the animals were hypertensive, demonstrated marked reductions in renal haemodynamics while fluid excretion was relatively normal. The data from the current studies show that, although there was a slight elevation in blood pressure, renal blood flow and glomerular filtration were markedly lower when compared to previous studies (5, 18). Although urine flow and sodium excretion in absolute terms was relatively normal, when considered as fractional sodium excretion it was some two to three times higher than observed in normal animals under identical conditions (17). It is these vascular and excretory levels which mean that the animals are in a state of reduced nephron function which can be considered equivalent to chronic renal failure.

The data from the vehicle group showed that renal haemodynamic and tubular function was stable over the time course of the study for both the innervated and denervated kidneys and, moreover, a denervation diuresis and natriuresis was present (20). It was apparent that administration of nitrendipine reduced blood pressure but caused a slight rise in renal blood flow, and even though filtered load remained constant, there was a marked natriuresis and diuresis again in both the innervated and denervated kidneys. The fact that there was an increase in fractional sodium excretion would indicate that there was a change in tubular handling of sodium but how this might occur is unclear. However, one possibility is that there was a dilatation within the kidney, as reflected by the increase in renal blood flow at a time when blood pressure was reduced, which would allow greater transmission of pressure into the renal interstitium, thereby decreasing sodium reabsorption (21). Indeed, in preliminary studies we have shown that nitrendipine increases renal interstitial pressure in normal Wistar rats (22). The vascular and tubular responses reported in this state of chronic renal failure are comparable to those obtained in normal kidneys. Thus, it would suggest that when this class of compound is given to chronic renal failure patients, there could be beneficial effects of not only reducing blood pressure but of assuring enhanced mobilisation of fluid. It maybe that these antihypertensive and excretory effects could help explain the slowing in the progression of the disease process when calcium channel antagonists are given (13, 23).

There is now a large body of evidence (20) providing strong support for the renal sympathetic nerves acting on vascular and tubular elements of the kidney to cause renal vasoconstriction, renin release and tubular reabsorption of sodium at the proximal tubule and thick limb of the ascending loop of Henle.

Thus, when the renal nerves were sectioned, a denervation diuresis and natriuresis became apparent, although this was somewhat smaller than had been reported previously, under comparable conditions (24). This would suggest that there was a basal tone of sympathetic drive under the conditions of the study. Our previous report in normal rats demonstrated that direct electrical stimulation of the renal nerves, having minimal effects on renal haemodynamics, caused both a sodium as well as calcium retention, compatible with a direct action on tubular reabsorptive processes (5). Moreover, this tubular action of the renal nerves appeared to be mediated *via* α_1-adrenoceptors (25) which seem to be of the α_{1A}-subtype (26). These previous studies had also found that in the presence of the calcium channel antagonist, nitrendipine, the ability of the renal nerves to cause an antinatriuresis and antidiuresis was preserved (25). It was evident from the current study, using the chronic renal failure rats, that the kidneys were under basal tonic influence of the renal nerves, since there was denervation diuresis and natriuresis. However, even though the renal nerves were stimulated effectively, as shown by the significant and reversible fall in renal blood flow, there was no meaningful changes in either water or sodium excretion. Furthermore, the pattern of these vascular and tubular responses were unaffected by the administration of nitrendipine. The reasons underlying this observation are not clear and a number of possibilities exist. Firstly, it may be that the adrenoceptor density at the tubular level is reduced under these conditions; secondly, that in chronic renal failure, two different populations of adrenoceptors become apparent (27); thirdly, a raised rate of fluid reabsorption by the remaining functional nephrons is relatively insensitive to adrenergic control. It is clear that each of these options need to be explored.

This study, undertaken in a rat model of chronic renal failure, shows that the compromised kidneys remain sensitive to the calcium channel antagonists in that they vasodilate and undergo a natriuresis and diuresis in response to the compounds. Moreover, the neural control of the tubular reabsorptive function appears defective and is not modulated by the calcium channel antagonist. However it needs to be stressed that these were acute experiments in anaesthesised animals.

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Author's address: Edward J. Johns, Department of Physiology, The Medical School, University of Birmingham, Birmingham B15 2TT, United Kingdom.