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A study in the rat of the renal actions of nitrendipine and diltiazem on the adrenergic regulation of calcium and sodium reabsorption

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1 In pentobarbitone-anaesthetized rats, intravenous administration of diltiazem at 5 $\mu g\,kg^{-1}\,min^{-1}$ did not change blood pressure or renal blood flow but increased glomerular filtration rate by approximately 16%, urine flow by 85%, calcium excretion by 151% and absolute and fractional sodium excretions by 100% and 69%, respectively. A similar pattern of responses was obtained in renally denervated animals, except that calcium excretion did not change statistically. Diltiazem given at 20 $\mu g\,kg^{-1}\,min^{-1}$ into renally innervated and denervated groups of animals depressed blood pressure between 15–17 mmHg but had no effect on renal haemodynamic or tubular function.

2 Nitrendipine administered at 0.5 $\mu g\,kg^{-1}\,min^{-1}$ to renally innervated and denervated animals significantly depressed blood pressure in intact animals by 6 mmHg and in both groups did not change renal haemodynamics but caused similar increases in urine flow of between 79–98%, calcium excretion of between 87 and 125%, absolute sodium excretion of between 108 and 140% and fractional sodium excretion of between 83 and 170%. Infusion of nitrendipine at 1.0 $\mu g\,kg^{-1}\,min^{-1}$ into intact or renally denervated animals decreased blood pressure by 18–20 mmHg and increased urine flow by 84–111%, calcium excretion by 85%, absolute sodium excretion by 81–137% and fractional sodium excretion by 52–102%.

3 Stimulation of the renal nerves at low frequencies (0.8 to 1.5 Hz) caused minimal changes in renal haemodynamics but decreased urine flow by 27%, calcium excretion by 35%, absolute and fractional sodium excretions 32% and 36%, respectively. In different groups of animals given either diltiazem at 20 $\mu g\,kg^{-1}\,min^{-1}$ or nitrendipine at 0.5 $\mu g\,kg^{-1}\,min^{-1}$ or 1.0 $\mu g\,kg^{-1}\,min^{-1}$, a similar degree of renal nerve stimulation caused an identical pattern of excretory responses of similar magnitude to those obtained in the absence of drug.

4 The calciuretic, diuretic and natriuretic activities of diltiazem and nitrendipine were not dependent on renal nerves and probably represented a direct action on the tubular reabsorptive processes of these ions. The renal nerve-induced increases in tubular calcium and sodium reabsorption indicate that these α-adrenoceptor-mediated responses are not dependent on the inward movement of calcium

Introduction

There are a number of reports which show that the calcium entry blocking drugs have an action on the kidney to increase the excretion of sodium and water in experimental animals (Bell & Lindner, 1984; Dietz *et al.*, 1983; Johns, 1985) and man (Zanchetti & Leonetti, 1985). The exact way in which these compounds alter the reabsorption of sodium and water is not known, but it could result from their action on renal haemodynamics, to increase renal blood flow, or glomerular filtration (Loutzenhiser & Epstein, 1985). A stronger possibility is that these compounds have a direct effect on active transport of ions by the tubular cells. Studies have been undertaken to locate a site of action along the tubule but there are inconsistent reports, with a micropuncture study by DiBona & Sawin (1983), using felodipine, suggesting a distal site, whereas a study with nicardipine (Abe *et al.*, 1983) indicated a more proximal site of action.

A further observation was that administration of methoxyverapamil (Brown & Churchill, 1983), or verapamil and nifedipine (Dietz *et al.*, 1983) increased the excretion of calcium again possibly reflecting an

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inhibition of the tubular reabsorptive processes for calcium. Calcium reabsorption from the glomerular filtrate takes place mainly at the proximal tubule (70%) with smaller fractions occurring at the loop of Henlé (15%) and the distal tubule and collecting duct (12%) (Dennis *et al.*, 1979). It is therefore possible that the blockade of calcium reabsorption by these drugs could occur at each of these sites.

An alternative consideration is that the calcium channel blocking drugs could interfere with the adrenergic regulation of sodium reabsorption by those renal sympathetic nerves making contact with the tubular epithelial cells (Barajas *et al.*, 1984). It is now recognized that activity within these nerves can directly stimulate the reabsorption of sodium ions by the tubular epithelial cells (DiBona, 1982) and in a recent study (Johns & Manitius, 1986a) calcium reabsorption was shown to be similarly regulated. The action of the sympathetic nerves on the epithelial cells involves α-adrenoceptors (Hesse & Johns, 1985) and the possibility arises that the calcium channel blocking drugs, which have the functional properties of α-adrenoceptor antagonists, could decrease the effectiveness of the renal nerves.

The intention of the present study was to examine the action of two chemically dissimilar calcium entry blocking drugs, diltiazem and the newly developed compound, nitrendipine (Scriabine *et al.*, 1984), on the handling of calcium and sodium by the kidney. This was done, firstly, by acutely administering the compounds into groups of animals which were either intact or had undergone acute renal denervation, and secondly, by determining the responses in calcium and sodium output when the renal nerves were stimulated at low frequencies causing undetectable changes in renal haemodynamics.

Methods

Male Sprague-Dawley rats (380–420 g) were anaesthetized with sodium pentobarbitone, 60 mg kg^{-1} intraperitoneally, and supplemental doses of 0.6 mg were administered every 15–20 min. The right carotid was cannulated for blood pressure measurements (a Statham P23ID pressure transducer connected to a Grass model 7D polygraph) and collection of blood samples. A cannula was placed in the jugular vein to allow infusion of saline (NaCl 150 mM) which was given at 6.0 ml h^{-1} for the duration of the experiment. The kidney was prepared for renal function measurements as previously described (Herod & Johns, 1985).

Renal function measurements

At the end of surgery 2 ml of saline containing inulin (10 mg ml^{-1}) was given intravenously over 2 min and the saline infusion changed to one containing inulin, 10 mg ml^{-1} which was infused for the remainder of the experiment. Two hours were allowed for recovery from surgery before measurements were started.

Experimental protocols

Renal actions of diltiazem and nitrendipine: the experimental protocal consisted of four 20 min clearance periods, two before and two after drug administration. On completion of the first two periods the infusion was changed to saline containing one of the drugs and 30 min later the final pair of clearance periods were taken.

Renal actions of diltiazem and nitrendipine: the experimental protocol consisted of four 20 min clearance and two after the third period during which the renal nerves were electrically stimulated. The renal nerves were stimulated at rates which were just sub-threshold for causing changes in renal blood flow and this required frequencies of between 0.8 and 1.5 Hz at 15 V and 0.2 ms duration. Each of the drugs was administered as a constant infusion which began 30 min before the start of the first clearance period. No urine sample was collected during the first 5 min of renal nerve stimulation or for the 5 min immediately after cessation of stimulation in order to allow pre-formed urine to clear from the cannula.

Arterial blood samples (0.35 ml) were taken at the beginning and end of the first and second pair of clearance periods, immediately centrifuged, the plasma removed for storage in the deep freeze and returned to the animal as soon as possible.

Inulin, in deproteinised plasma and urine samples (Somogyi, 1930), was estimated according to the method of Bojesen (1952), as described by Johns *et al.* (1976), and glomerular filtration rate calculated. Urinary and plasma levels of sodium were measured with a Beckman flame photometer and urinary calcium concentrations were estimated with a Perkin-Elmer 2380 atomic absorption spectrophotometer.

Drugs

A stock solution of diltiazem, 1 mg ml^{-1} in saline, was freshly prepared every 96 h and stored in the fridge. Dilutions of this stock were made into the saline infusion such that it was delivered at a rate of 5 or 20 μg kg^{-1} min^{-1}. A stock solution of nitrendipine at 5 mg ml^{-1} was prepared by dissolving the compound in 1:1 glycofural:ethanol mixture and aliquots were stored deep frozen for no longer than 4 days. This

stock was diluted into the saline infusion so that it was delivered at a rate of either 0.5 or 1.0 $\mu g\,kg^{-1}\,min^{-1}$.

Statistics

The absolute and percentage changes quoted in the text represent a mean of the individual changes recorded in each animal. In the drug administration experiments a mean value of the two clearances before the drug was compared with a mean value of the two clearances obtained during the infusion of the drug. The renal responses to nerve stimulation were measured by taking the average value of the two clearances before and the two after stimulation and comparing it to the value obtained during nerve stimulation. All data are expressed as means ± s.e.mean. The paired Student's t test was used for intra-group analysis and the unpaired Student's t test for inter-group analysis. Differences between means were considered significant at the 5% level.

Results

Renal actions of diltiazem

Administration of diltiazem at 5 $\mu g\,kg^{-1}\,min^{-1}$ into the renally innervated animals (Table 1) had no effect on either blood pressure or renal blood flow but there were significant increases in glomerular filtration rate of 16% ($P<0.05$), urine flow of 85% ($P<0.001$), calcium excretion of 151% ($P<0.001$) and absolute and fractional sodium excretions of 100% ($P<0.001$) and 69% ($P<0.01$), respectively. In denervated animals (Table 1), infusion of diltiazem at 5 $\mu g\,kg^{-1}\,min^{-1}$ did not change blood pressure or renal blood flow but significantly increased glomerular filtration rate by 12% ($P<0.05$), urine flow by 82% ($P<0.05$) and absolute and fractional sodium excretions of 126% ($P<0.01$) and 113% ($P<0.05$), respectively; however, calcium excretion did not change statistically. The magnitude and pattern of changes in all variables were the same whether the renal nerves were present or not, except calcium excretion in the denervated animals which was significantly ($P<0.02$) less than when the nerves were present.

Administration of diltiazem, 20 $\mu g\,kg^{-1}\,min^{-1}$, into intact animals (Table 2) caused a significant ($P<0.05$) reduction in blood pressure of 15 mmHg. Both renal blood flow and glomerular filtration rate remained stable while there were no statistical changes in the excretion of water, calcium and sodium. Infusion of diltiazem at 20 $\mu g\,kg^{-1}\,min^{-1}$ into the denervated animals depressed blood pressure significantly ($P<0.01$) by 17 mmHg with no change in renal blood flow or glomerular filtration rate while the excretory rates of water, calcium and sodium were very variable and did not reach statistical significance. The pattern of blood pressure and renal responses caused by the high doses of diltiazem was very different from that observed at the lower dose of diltiazem irrespective of whether the nerves were present or not.

Renal actions of nitrendipine

Infusion of nitrendipine at 0.5 $\mu g\,kg^{-1}\,min^{-}$ into innervated animals (Table 3) caused a significant ($P<0.05$) reduction in blood pressure of 8 mmHg but did not change either renal blood flow or glomerular filtration rate; however, there were significant increases in urine flow of 79% ($P<0.05$), calcium excretion of 87% ($P<0.02$) and in absolute and

Table 1 Effect of diltiazem, 5 $\mu g\,kg^{-1}\,min^{-1}$ i.v., on blood pressure and renal function in animals with an innervated or denervated left kidney

	Innervated ($n=8$)		*Denervated* ($n=8$)	
	Basal	*Drug*	*Basal*	*Drug*
Blood pressure (mmHg)	130 ± 1	129 ± 3	117 ± 4	114 ± 5
Renal blood flow (ml $min^{-1}\,kg^{-1}$)	15.6 ± 0.7	15.2 ± 0.9	16.2 ± 1.7	17.0 ± 1.7
Glomerular filtration rate (ml $min^{-1}\,kg^{-1}$)	2.67 ± 0.20	3.12 ± 0.19*	3.04 ± 0.42	3.41 ± 0.50*
Urine flow (μl $min^{-1}\,kg^{-1}$)	69.4 ± 12.7	114.1 ± 12.3***	34.2 ± 7.3	61.8 ± 15.5*
Absolute calcium excretion (nmol $min^{-1}\,kg^{-1}$)	94.1 ± 27.6	203.5 ± 47.3***	60.0 ± 15.0	99.0 ± 27.0
Absolute sodium excretion (μmol $min^{-1}\,kg^{-1}$)	12.3 ± 2.0	21.7 ± 1.7	7.1 ± 1.5	13.1 ± 1.8***
Fractional sodium excretion (%)	3.59 ± 0.58	5.44 ± 0.51**	1.78 ± 0.37	3.60 ± 0.99*

The P values represent a comparison between the mean of the two clearances before and the mean of the two clearances obtained during the infusion of drug: *$P<0.05$; **$P<0.01$; ***$P<0.001$.

Table 2 Effect of diltiazem, 20 $\mu g\,kg^{-1}\,min^{-1}$ i.v., on blood pressure and renal function in animals with an innervated or denervated left kidney

	Innervated ($n = 8$)		*Denervated* ($n = 8$)	
	Basal	*Drug*	*Basal*	*Drug*
Blood pressure (mmHg)	120 ± 3	105 ± 7*	132 ± 5	115 ± 5**
Renal blood flow ($ml\,min^{-1}\,kg^{-1}$)	14.4 ± 0.9	13.9 ± 1.4	14.4 ± 0.8	14.9 ± 0.8
Glomerular filtration rate ($ml\,min^{-1}\,kg^{-1}$)	2.87 ± 0.37	2.13 ± 0.26	3.08 ± 0.35	3.25 ± 0.29
Urine flow ($\mu l\,min^{-1}\,kg^{-1}$)	43.2 ± 9.3	49.8 ± 10.3	36.6 ± 4.6	50.7 ± 8.3
Absolute calcium excretion ($nmol\,min^{-1}\,kg^{-1}$)	62.0 ± 13.0	71.0 ± 19.0	58.0 ± 13.0	89.0 ± 24.0
Absolute sodium excretion ($\mu mol\,min^{-1}\,kg^{-1}$)	7.81 ± 1.71	9.91 ± 2.35	8.51 ± 1.71	13.32 ± 3.34
Fractional sodium excretion (%)	2.04 ± 0.61	2.57 ± 0.58	2.02 ± 0.28	2.94 ± 0.69

The P values represent a comparison between the mean of the two clearance periods before and the mean of the two clearance periods obtained during drug infusion: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 3 Effect of nitrendipine, 0.5 $\mu g\,kg^{-1}\,min^{-1}$ i.v., on blood pressure and renal function in animals with an innervated or denervated left kidney

	Innervated ($n = 7$)		*Denervated* ($n = 8$)	
	Basal	*Drug*	*Basal*	*Drug*
Blood pressure (mmHg)	124 ± 3	116 ± 4*	121 ± 2	116 ± 2
Renal blood flow ($ml\,min^{-1}\,kg^{-1}$)	15.5 ± 1.0	16.1 ± 1.6	12.1 ± 1.3	13.0 ± 1.5
Glomerular filtration rate ($ml\,min^{-1}\,kg^{-1}$)	2.86 ± 0.25	2.90 ± 0.22	2.93 ± 0.39	3.25 ± 0.32
Urine flow ($\mu l\,min^{-1}\,kg^{-1}$)	36.1 ± 3.8	66.7 ± 12.7***	46.6 ± 4.5	90.1 ± 12.9**
Absolute calcium excretion ($nmol\,min^{-1}\,kg^{-1}$)	59.0 ± 10.0	105.0 ± 19.0*	75.0 ± 11.0	170.0 ± 31.0**
Absolute sodium excretion ($\mu mol\,min^{-1}\,kg^{-1}$)	7.63 ± 1.35	16.06 ± 2.07**	7.76 ± 0.84	17.32 ± 2.44**
Fractional sodium excretion (%)	1.66 ± 0.23	3.99 ± 0.62**	1.92 ± 0.18	3.83 ± 0.58*

The P values represent a comparison between the mean of the two clearance periods before and the mean of the two clearance periods obtained during the drug infusion: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 4 Effect of nitrendipine, 1.0 $\mu g\,kg^{-1}\,min^{-1}$ i.v., on blood pressure and renal function in animals with an innervated or denervated left kidney

	Innervated ($n = 8$)		*Denervated* ($n = 8$)	
	Basal	*Drug*	*Basal*	*Drug*
Blood pressure (mmHg)	122 ± 3	104 ± 4**	135 ± 4	115 ± 5***
Renal blood flow ($ml\,min^{-1}\,kg^{-1}$)	15.6 ± 1.5	16.2 ± 1.0	15.0 ± 1.1	14.4 ± 0.9
Glomerular filtration rate ($ml\,min^{-1}\,kg^{-1}$)	2.42 ± 0.36	3.09 ± 0.32	3.20 ± 0.50	3.51 ± 0.42
Urine flow ($\mu l\,min^{-1}\,kg^{-1}$)	48.1 ± 7.2	81.1 ± 8.1*	41.1 ± 9.2	74.3 ± 9.7***
Absolute calcium excretion ($nmol\,min^{-1}\,kg^{-1}$)	68.0 ± 17.0	109.0 ± 18.0*	78.7 ± 18.2	130.5 ± 22.2***
Absolute sodium excretion ($\mu mol\,min^{-1}\,kg^{-1}$)	9.83 ± 2.11	15.4 ± 1.8*	8.0 ± 2.3	14.7 ± 2.6**
Fractional sodium excretion (%)	2.79 ± 0.67	3.99 ± 0.79*	1.51 ± 0.81	2.88 ± 0.43**

The P values represent a comparison between the mean of the two clearance periods before and the mean of the two clearance periods obtained during drug infusion: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 5 Effect of low-frequency renal nerve stimulation on blood pressure and renal function in the absence and presence of diltiazem, 20 $\mu g\,kg^{-1}\,min^{-1}$ i.v.

	Saline ($n = 6$)			*Diltiazem* ($n = 7$)		
	Control	*Experimental*	*Recovery*	*Control*	*Experimental*	*Recovery*
Blood pressure (mmHg)	133 ± 5	132 ± 5	139 ± 7	107 ± 4	102 ± 5	98 ± 5
Renal blood flow ($ml\,min^{-1}\,kg^{-1}$)	15.9 ± 1.3	15.8 ± 1.3	16.2 ± 1.6	11.4 ± 0.7	10.7 ± 1.1	11.1 ± 0.9
Glomerular filtration rate ($ml\,min^{-1}\,kg^{-1}$)	3.20 ± 0.14	3.27 ± 0.27	3.25 ± 0.50	2.44 ± 0.31	1.97 ± 0.50	3.06 ± 0.26
Urine flow ($\mu l\,min^{-1}\,kg^{-1}$)	51.2 ± 6.1	38.5 ± 6.3**	53.1 ± 10.4	60.7 ± 9.3	31.9 ± 5.7***	65.2 ± 7.9
Absolute calcium excretion ($nmol\,min^{-1}\,kg^{-1}$)	113.9 ± 16.7	84.1 ± 15.4**	141.4 ± 26.7	93.5 ± 21.1	38.7 ± 9.3**	62.4 ± 10.0
Absolute sodium excretion ($\mu mol\,min^{-1}\,kg^{-1}$)	12.9 ± 1.4	9.12 ± 1.90***	12.7 ± 2.6	11.5 ± 1.9	4.84 ± 1.2***	8.9 ± 1.6
Fractional sodium excretion (%)	2.74 ± 0.35	2.07 ± 0.58***	3.27 ± 1.09	3.53 ± 0.87	1.84 ± 0.54**	2.93 ± 0.81

The P values represent a comparison of the mean of the four clearances of the control and recovery periods with the value obtained during the period of renal nerve stimulation (experimental): *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 6 Effect of low-frequency renal nerve stimulation on blood pressure and renal function in the absence and presence of nitrendipine

	Nitrendipine (0.5 $\mu g\,kg^{-1}\,min^{-1}$) ($n = 6$)			*Nitrendipine* (1.0 $\mu g\,kg^{-1}\,min^{-1}$) ($n = 7$)		
	Control	*Experimental*	*Recovery*	*Control*	*Experimental*	*Recovery*
Blood pressure (mmHg)	110 ± 1	109 ± 2	109 ± 3	109 ± 3	104 ± 6	105 ± 6
Renal blood flow ($ml\,min^{-1}\,kg^{-1}$)	15.8 ± 1.6	14.6 ± 1.4*	15.8 ± 1.3	14.1 ± 1.6	12.9 ± 1.2	13.2 ± 1.2
Glomerular filtration rate ($ml\,min^{-1}\,kg^{-1}$)	3.06 ± 0.26	3.00 ± 0.26	2.98 ± 0.27	3.60 ± 0.15	3.35 ± 0.35	3.49 ± 0.26
Urine flow ($\mu l\,min^{-1}\,kg^{-1}$)	73.4 ± 8.2	45.7 ± 9.8**	79.3 ± 10.7	69.9 ± 15.7	41.2 ± 12.2*	60.4 ± 16.7
Absolute calcium excretion ($nmol\,min^{-1}\,kg^{-1}$)	169.7 ± 23.5	108.2 ± 25.0**	184.7 ± 37.3	108.9 ± 35.4	77.6 ± 30.7*	113.5 ± 45.8
Absolute sodium excretion ($\mu mol\,min^{-1}\,kg^{-1}$)	17.1 ± 2.5	10.1 ± 1.8**	17.8 ± 3.1	15.8 ± 4.8	8.9 ± 3.5*	14.0 ± 5.9
Fractional sodium excretion (%)	3.73 ± 0.56	2.11 ± 3.88**	4.24 ± 0.88	2.99 ± 0.90	1.88 ± 0.74*	2.68 ± 0.93

The P values represent a comparison of the mean of the four clearances of the control and recovery periods with the value obtained during the period of renal nerve stimulation (experimental): *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

fractional sodium excretions of 108% ($P<0.01$) and 170% ($P<0.01$), respectively. In denervated animals, administration of the low dose of nitrendipine (Table 3) did not change blood pressure, had no effect on either renal blood flow or glomerular filtration rate and significantly increased urine flow by 98% ($P<0.02$), calcium excretion by 125% ($P<0.01$) and absolute and fractional sodium excretions by 140% ($P<0.01$) and 83% ($P<0.02$), respectively. The pattern and magnitude of the renal responses to nitrendipine at 0.5 $\mu g\,kg^{-1}\,min^{-1}$ were the same whether the renal nerves were intact or not.

In innervated animals administration of nitrendipine at 1.0 $\mu g\,kg^{-1}\,min^{-1}$ (Table 4) decreased blood pressure by 18 mmHg, had no effect on either renal blood flow or glomerular filtration rate, but significantly increased urine flow by 84% ($P<0.02$), calcium excretion by 85% ($P<0.05$) and absolute and fractional sodium excretions by 81% ($P<0.05$) and 52% ($P<0.05$), respectively. In denervated animals, the high dose of nitrendipine (Table 4) decreased blood pressure by 20 mmHg ($P<0.001$) but had no effect on either renal blood flow or glomerular filtration rate and increased urine flow by 111% ($P<0.001$), calcium excretion by 85% ($P<0.01$) and absolute and fractional sodium excretions by 137% ($P<0.001$) and 102% ($P<0.01$), respectively, which were identical in pattern and magnitude to those in the innervated animals. The renal responses observed during the infusion of 0.5 and 1.0 $\mu g\,kg^{-1}\,min^{-1}$ nitrendipine were not statistically different.

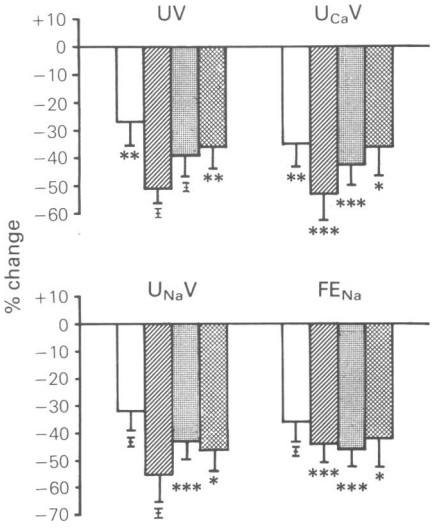

Figure 1 Percentage changes occurring in urine flow (UV), calcium excretion ($U_{Ca}V$), absolute sodium excretion ($U_{Na}V$) and fractional sodium excretion (FE_{Na}) in response to low frequency stimulation (0.8–1.5 Hz) renal nerve stimulation alone (open columns, $n=6$) and during the infusion of diltiazem, at 20 $\mu g\,kg^{-1}\,min^{-1}$ (hatched columns, $n=7$) and nitrendipine, at 0.5 (stippled columns, $n=7$) and 1.0 $\mu g\,kg^{-1}\,min^{-1}$ (cross hatched columns, $n=6$). $^{*}P<0.05$; $^{**}P<0.02$; $^{***}P<0.01$; $\dagger P<0.001$. The percentage changes represent a mean of the individual changes recorded in each animal and were calculated by taking the mean of the two clearances before and the two following renal nerve stimulation and comparing it to the value obtained during stimulation.

Renal nerve stimulation

Saline infusion Blood pressure remained at a stable level throughout the experimental period and the renal nerve stimulation did not significantly affect either renal blood flow or glomerular filtration (Table 5) while there were significant reductions in urine flow of 27% ($P<0.02$), calcium excretion by 35% ($P<0.02$) and absolute and fractional sodium excretions by 32% ($P<0.001$) and 36% ($P<0.001$), respectively.

Diltiazem infusion In animals given diltiazem at 20 $\mu g\,kg^{-1}\,min^{-1}$ (Table 5), blood pressure was significantly ($P<0.01$) lower than in animals not given the drug but remained stable throughout the experiment. During renal nerve stimulation, neither renal blood flow nor glomerular filtration rate changed; however, there were significant reductions in urine flow of 51% ($P<0.001$), calcium excretion of 53% ($P<0.01$) and absolute and fractional sodium excretions of 55% ($P<0.001$) and 44% ($P<0.01$). These responses were not significantly different from those obtained in the saline-infused animals.

Nitrendipine infusion. In the group of animals given nitrendipine at 0.5 $\mu g\,kg^{-1}\,min^{-1}$ (Table 6), blood pressure was significantly ($P<0.01$) less than those animals infused with saline and remained stable during the experiment. Renal nerve stimulation in the presence of nitrendipine, 0.5 $\mu g\,kg^{-1}\,min^{-1}$, caused a significant ($P<0.02$) reduction in renal blood flow of 7% with no change in glomerular filtration rate while there were significant reductions in urine flow of 39% ($P<0.001$), calcium excretion of 42% ($P<0.01$) and absolute and fractional sodium excretions of 43% ($P<0.01$) and 46% ($P<0.01$), respectively. These responses could not be distinguished from those obtained in the presence of diltiazem, 20 $\mu g\,kg^{-1}\,min^{-1}$, or in the absence of drug.

The blood pressure of animals given nitrendipine at 1.0 $\mu g\,kg^{-1}\,min^{-1}$ (Table 6) was significantly ($P<0.01$) lower than in those not receiving a drug (Table 5), but it remained stable throughout the experiment. Renal nerve stimulation in these animals did not change renal haemodynamics but significantly decreased

urine flow by 36% ($P<0.02$), calcium excretion by 36% ($P<0.05$) and absolute and fractional sodium excretions by 46% ($P<0.05$) and 42% ($P<0.05$), respectively, which were responses not significantly different from those observed with either nitrendipine at 0.5 $\mu g\,kg^{-1}\,min^{-1}$, diltiazem at 20 $\mu g\,kg^{-1}\,min^{-1}$ (Table 5), or in those in which the animals were not infused with drug (Table 5). A comparison of these responses is shown in Figure 1.

Discussion

The aim of this work was to describe the action of calcium channel blockade on renal haemodynamics and function, particularly with regard to the regulation of calcium handling by the kidney and the adrenergic control of calcium reabsorption. Nitrendipine and diltiazem were used at low doses that had minimal effects on blood pressure and higher doses that caused depressions of blood pressure of between 15–20 mmHg.

Renal blood flow remained constant throughout the experiments at both doses of nitrendipine and diltiazem. There have been a number of reports of increases in blood flow in response to calcium channel blockade but this appears to be variable and dependent on the experimental conditions (Loutzenhiser & Epstein, 1985). In the present study, a stable renal blood flow in the face of a fall in blood pressure at the high doses of drugs would suggest that there had been a decrease in renal vascular resistance. The low dose of diltiazem caused a significant rise in glomerular filtration rate in both renally innervated and denervated animals which was similar to that observed previously in the rat (Johns, 1985) and by Yamaguchi *et al.* (1974) in the dog. The mechanisms responsible for this response are not clear but probably reflect changes in the relationship between resistances of the afferent and efferent arterioles such that glomerular filtration pressure rises. This action on glomerular filtration rate does seem to be a characteristic of diltiazem as it did not occur following the administration of either dose of nitrendipine when given under identical experimental conditions.

The calciuretic action of both doses of nitrendipine and the low dose of diltiazem has also been reported for methoxyverapamil (Brown & Churchill, 1983) and for verapamil and nifedipine (Dietz *et al.*, 1983) and therefore may be a common feature of calcium channel blocking drugs. These compounds might act on the calcium reabsorptive processes of the proximal tubule, the primary site of calcium reabsorption (Dennis *et al.*, 1979). It was unlikely that the increased calcium excretion was due to blockade of renal nerve activity as similar responses were recorded in both renally innervated and denervated animals. However, a number of possibilities could account for these responses; firstly, these compounds could block the movement of calcium ions from the tubular fluid into the cells; secondly, they could interfere with the calcium ATPase system at the tubular cells (Katz, 1986); thirdly, they could act indirectly via changes in sodium reabsorption which appears to provide, in part, the energy required to operate the sodium/calcium counter transport system (Taylor & Windhager, 1979).

At the high dose of diltiazem, calcium excretion did not change significantly which could have been the result of the marked fall in blood pressure directly inhibiting the calcium reabsorptive processes and thereby counteracting the calciuretic activity of the diltiazem on the kidney. If this was so, then the observation that nitrendipine at the high doses, which caused a similar reduction in blood pressure, still had a potent calciuretic effect would indicate that nitrendipine was relatively more effective on the tubular calcium reabsorptive processes than was diltiazem. The finding that the high dose of diltiazem did not change sodium and water excretion could, as with the calcium results, have been a consequence of the large fall in blood pressure as sodium reabsorption is known to be dependent on blood pressure (Roman & Cowley, 1985). Nitrendipine also had diuretic and natriuretic activities in renally innervated and denervated animals, the magnitude of which was the same whether the high or low dose of nitrendipine was given. At high doses, nitrendipine caused falls in blood pressure of between 18–20 mmHg but in spite of this there was still a doubling of water and sodium output demonstrating its potent tubular action.

The decreased calcium excretion during low frequency renal nerve stimulation has been taken to be an action of those renal nerves ending in close proximity to the tubular cells, mainly in the proximal tubule (Pelayo *et al.*, 1983) and the thick ascending limb of the loop of Henlé (DiBona & Sawin, 1982; Bencsath *et al.*, 1985), whereby they stimulate the processes for calcium reabsorption (Johns & Manitius, 1986a). The underlying mechanisms of this nerve-activated calcium reabsorption are unknown, but could be due to either a direct stimulation of the calcium ATPase or indirectly via stimulation of the sodium/potassium ATPase of the tubular cells (Taylor & Windhager, 1979).

Calcium channel blocking drugs are able to interfere with α-adrenoceptor responses which appear to be primarily an action on postsynaptic α_2-adrenoceptor sites (van Zwieten *et al.*, 1985). However, there is evidence that at vascular smooth muscle junctions, for example rat aorta and rabbit coronary artery (Vanhoutte & Rimele, 1982), and in spontaneously hypertensive rat models (Kazda *et al.*, 1985), that α_1-adren-

oceptor-mediated vasoconstrictor responses can be dependent on inward movement of calcium. Renal nerve-induced increases in tubular sodium reabsorption appear to be mediated by α_1-adrenoceptors in the dog (Osborn *et al.*, 1983), the rabbit (Hesse & Johns, 1985) as well as the rat (Johns & Manitius, 1986b) and the possibility existed that calcium channel blockers could interfere with neurotransmission at this specialized junction. However, the magnitude of the reduction in the calcium excretion due to the low frequency renal nerve stimulation was not altered by either dose of drug. Further, the degree of nerve-mediated antidiuresis and antinatriuresis was not altered following administration of the drugs which was similar to observations with nifedipine (Herod & Johns, 1985). Thus, renal nerve stimulation of tubular reabsorption of both calcium and sodium appeared not to be dependent on the inward movement of calcium at the neuro-epithelial cell junction. This situation is in contrast to that at renal vascular junctions as Mejia *et al.* (1984) found that adrenergically induced renal vasoconstriction in the rat could be prevented by administration of verapamil and diltiazem. The reasons why α-adrenoceptors mediating the action of the renal nerves at tubules and vasculature should be differentially affected by the calcium channel blocking drugs remains to be resolved.

The present study has shown that administration of diltiazem and nitrendipine, had small or minimal actions on renal haemodynamics but caused large increases in the output of water, calcium and sodium. This tubular action occurred irrespective of whether the renal nerves were present or not which would suggest that these responses represented a direct tubular effect. Low frequency renal nerve stimulation, causing minimal changes in renal haemodynamics, decreased water, calcium and sodium output and these responses were not changed by diltiazem or nitrendipine. These results show that renal nerve-induced reabsorption of calcium and sodium does not depend on activation of α-adrenoceptors dependent on the inward movement of calcium.

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