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A study of the renal actions of amlodipine in the normotensive and spontaneously hypertensive rat

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1 Normotensive Sprague-Dawley and spontaneously hypertensive rats anaesthetized with sodium pentobarbitone were used to determine the systemic and renal actions of amlodipine, a new calcium channel blocking drug.

2 Amlodipine, 200 $\mu g\,kg^{-1}$ plus 50 $\mu g\,kg^{-1}\,h^{-1}$, decreased blood pressure by 12 ± 3 mmHg in normotensive rats, although the fall was not statistically significant in the hypertensive rats; did not change renal haemodynamics and caused significant increases in urine flow, absolute and fractional sodium excretions of 70%, 91% and 113%, respectively, in normotensive rats and 65%, 91% and 96%, respectively in hypertensive rats. Fractional lithium excretion was unchanged in the normotensive rats but increased by 28% in the hypertensive animals while absolute fluid reabsorption in the proximal tubule did not change in either group. Absolute water and sodium reabsorption in the segments beyond the proximal tubule were unchanged in the normotensive rats but increased in the hypertensive animals by 24% and 22%, respectively, while fractional sodium excretion in this portion of the nephron increased by 88% and 51% in the normotensive and hypertensive rats, respectively.

3 Amlodipine, 400 $\mu g\,kg^{-1}$ plus 100 $\mu g\,kg^{-1}\,h^{-1}$, decreased blood pressure by 12 ± 4 mmHg in the normotensive and by 27 ± 5 mmHg in the hypertensive rats. Renal blood flow was not changed in either group of rats and glomerular filtration rate increased by 25% in the spontaneously hypertensive animals. There were significant increases in urine flow, absolute and fractional sodium excretions of 105%, 145% and 142%, respectively, in the normotensive rats and 224%, 421% and 259%, respectively, in the hypertensive rats. Fractional lithium excretion was elevated by 29% and 38%, in the normotensive and hypertensive rats, respectively, but absolute fluid reabsorption at the proximal tubule remained unchanged. At the same time there were significant increases in absolute water and sodium reabsorption beyond the proximal tubule of 26% and 18%, respectively, in the normotensive animals and of 63% and 60%, respectively, in the hypertensive animals. Fractional excretion of water and sodium in the nephron regions after the proximal tubule were increased by 55% and 88%, respectively, in the normotensive rats and by 84% and 121%, respectively, in the hypertensive rats.

4 These doses of amlodipine caused modest reductions in blood pressure, minimal changes in renal haemodynamics and a natriuresis and diuresis. Proximal sodium and water reabsorption was not affected by the drug and it is suggested that the changes in tubular fluid handling were compatible with depression of reabsorption further along the tubule.

Introduction

The calcium channel blocking drugs have now been recognised as antihypertensive agents whose primary action is one of decreasing cardiac contractility and causing dilatation of vascular smooth muscle (Godfraind, *et al.*, 1986). These drugs have the potential of acting on both the renal vasculature and tubules. The renal haemodynamic responses to the calcium channel blocking drugs are highly variable (Loutzenhiser & Epstein, 1985) and dependent on the basal vasoconstrictor tone of the kidney which is dictated by neural and circulating agents but it is clear that in the presence of these vasodepressor compounds glomerular filtration rate is well preserved.

Evidence is accumulating that calcium channel blocking drugs increase the excretion of sodium and water in normotensive (Ene *et al.*, 1985) and hyper-

tensive man (Zanchetti & Leonetti, 1985). Experimental studies have shown that acute administration of calcium channel blockers in the dog (Dietz *et al.*, 1983; Bell & Lindner, 1984) or rat (Brown & Churchill, 1983; Johns, 1985) caused large increases in the output of sodium and water which occurred without changes in either renal blood flow or glomerular filtration rate. Similar natriuretic and diuretic properties have been reported for the calcium channel blocker, felodipine, when given to spontaneously hypertensive rats (Nordlander *et al.*, 1985). Relatively little is known of the action of the drugs in the genetic models of hypertension and no comparison with those obtained in normotensive animals has yet been undertaken.

The underlying mechanisms and site of action responsible for the natriuresis and diuresis induced by the calcium channel blockers are unknown. Our own studies have shown that these drugs are equally effective in renally denervated kidneys (Johns, 1985) and do not interfere with the adrenergic regulation of sodium handling by the renal tubules (Herod & Johns, 1985; Johns & Manitius, 1986). The study of DiBona & Sawin (1984), using micropuncture techniques in the rat, indicated that felodipine inhibited sodium reabsorption at the late distal tubule. In contrast, Abe and co-workers (1983) using whole kidney techniques in the dog, concluded that nicardipine inhibited sodium reabsorption at both proximal and distal tubules while Wallia *et al.* (1985) provided evidence for a proximal tubular site of action for nitrendipine in man. Further, there appears to be no reports of depressed sodium transport by the calcium channel blockers in *in vitro* tubule segments or membranes.

The aim of the present study was two fold; first, to examine the actions of calcium channel blockade on renal haemodynamics and tubular function in the spontaneously hypertensive rats and compare the responses with those of normotensive animals; second, to attempt to determine the site of action of the calcium channel blocking drug along the tubules. Normotensive and spontaneously hypertensive rats were given increasing doses of the newly developed, calcium channel-blocking drug, amlodipine (Burges *et al.*, 1987) and proximal tubular fluid uptake was estimated by the lithium clearance technique (Thomsen, 1984).

Methods

Male albino Sprague-Dawley rats (300–425 g) were obtained from the Departmental Animal House and the spontaneously hypertensive rats (300–400 g) were purchased (Olac, Bicester) at 8 weeks of age and maintained in the Animal House until use at a final age of between 16–20 weeks. Animals were anaesthetized with sodium pentobarbitone, $60\,mg\,kg^{-1}$, i.p., and supplemented with $15\,mg\,kg^{-1}\,h^{-1}$, i.v., throughout the experiment. Cannulation of the right carotid artery allowed removal of blood samples and measurement of blood pressure (Statham P23ID pressure transducer attached to a Grass Model 7D polygraph). The left jugular vein was cannulated and immediately an infusion was begun at $6\,ml\,h^{-1}$ of a solution containing 150 mmol NaCl and 16 mmol LiCl which was continued until the end of the experiment.

The left kidney was exposed by a mid-line abdominal incision, the ureter cannulated for urine collection and the renal artery cleared so that an electromagnetic flow probe (Carolina EP100 series) could be fitted in order to measure renal blood flow directly (Carolina FM501 flowmeter linked to a Grass model 7D polygraph). By use of a Zeiss model 212 surgical microscope, all nerves traversing to the kidney were isolated and sectioned.

Renal function measurement

As soon as surgery was completed, 2 ml of saline containing LiCl (16 mmol) and inulin ($10\,mg\,ml^{-1}$) was given intravenously over 1 min and the infusion changed to one containing inulin ($10\,mg\,ml^{-1}$) which was infused for the duration of the experiment. Measurements were begun 2 h later.

The experiment consisted of 4 clearance periods of either 15 min or 20 min duration, two before and two after the drug administration. Once the first two clearances had been completed a bolus dose of amlodopine was given and the infusion changed to one which contained the appropriate concentration of drug. The second set of clearances were taken 15 min later.

Arterial blood samples (0.6 ml) were taken at the beginning and end of each pair of clearances, immediately centrifuged for 2 min, the plasma removed for storage in the deep-freeze and the red cells resuspended in an equal volume of saline to enable them to be returned to the animal as quickly as possible. Mean blood pressure and renal blood flow were measured over each clearance period by use of a BBC microcomputer and a Unilab interface linked to a Torch Z80 disc drive which was programmed to accept input data from the Grass polygraph (Emmerson & Johns, 1986). Urinary and plasma inulin concentrations were estimated as previously described (Johns *et al.*, 1976) and glomerular filtration rate was calculated as the clearance of inulin (Arundell & Johns, 1982). Sodium and lithium concentrations in plasma and urine was measured by emission spectroscopy (Corning 410C).

Table 1 Effect of amlodipine on blood pressure and renal function in normotensive rats

	Vehicle ($n = 7$)		*Amlodipine* ($200\,\mu g\,kg^{-1}$) ($n = 7$)		*Amlodipine* ($400\,\mu g\,kg^{-1}$) ($n = 8$)	
	Before	*After*	*Before*	*After*	*Before*	*After*
BP (mmHg)	132 ± 4	132 ± 5	145 ± 3	134 ± 5**	138 ± 5	126 ± 9*
RBF ($ml\,min^{-1}\,kg^{-1}$)	16.6 ± 2.0	16.5 ± 2.1	17.8 ± 1.9	18.4 ± 2.0	19.1 ± 1.9	20.6 ± 1.8
GFR ($ml\,min^{-1}\,kg^{-1}$)	3.30 ± 0.43	3.46 ± 0.40	4.36 ± 0.51	4.15 ± 0.66	4.25 ± 0.44	4.17 ± 0.27
UV ($\mu l\,min^{-1}\,kg^{-1}$)	57.6 ± 16.0	59.7 ± 15.6	68.5 ± 20.8	103.0 ± 25.3**	59.7 ± 10.5	111.0 ± 13.8***
$U_{Na}V$ ($\mu mol\,min^{-1}\,kg^{-1}$)	17.2 ± 5.7	16.7 ± 5.9	14.1 ± 3.9	23.1 ± 5.3**	13.1 ± 2.6	28.4 ± 3.9***
Fe_{Na} (%)	3.31 ± 0.94	3.29 ± 1.00	2.37 ± 0.64	4.46 ± 1.11*	2.44 ± 0.48	5.29 ± 0.73***
FE_{Li} (%)	25.7 ± 4.6	26.3 ± 3.7	17.5 ± 2.2	22.8 ± 3.6	20.5 ± 1.5	26.4 ± 2.0***

The P values represent a comparison between a mean of the two clearances before and the two clearances following administration of the drug or vehicle. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. BP = blood pressure; RBF = renal blood flow; GFR = glomerular filtration rate; UV = urine flow; $U_{Na}V$ = absolute sodium excretion; FE_{Na} = fractional sodium excretion; FE_{Li} = fractional lithium excretion.

Experimental groups

Groups of normotensive and hypertensive animals were subjected to one of the following regimes: (a) vehicle infusion: immediately following the first two clearances a bolus of saline (0.1 ml $100\,g^{-1}$ body wt, i.v.) was given and the infusion continued; (b) amlodipine, low dose: after the completion of the first pair of clearances a bolus dose of amlodipine, $200\,\mu g\,kg^{-1}$, i.v. in 0.1 ml $100\,g^{-1}$ body weight, was given and the infusion changed to one containing amlodipine such that it was delivered at $50\,\mu g\,kg^{-1}\,h^{-1}$; (c) amlodipine, high dose: after the first two clearances a bolus of amlodipine, $400\,\mu g\,kg^{-1}$ i.v. in 0.1 ml $100\,g^{-1}$ body weight, was given and the infusion changed to one containing amlodipine such that it was delivered at $100\,\mu g\,kg^{-1}\,h^{-1}$.

A stock solution of amlopidine, $500\,\mu g\,ml^{-1}$ in saline, was kept refrigerated and prepared fresh every 3 days. The bolus injections were made by dilution of this stock in saline while the infusion was made by dilution of the stock with saline containing LiCl, pentobarbitone and inulin.

Calculation of data

Standard formulae were used to calculate the clearance of inulin (GFR), sodium (C_{Na}) and lithium (C_{Li}) and fractional excretion of sodium ($FE_{Na} = C_{Na}/GFR$). Thomsen (1984) has shown that C_{Li} is a measure of fluid delivery from the proximal tubule into the loop of Henlé and distal tubule. Consequently the following derivations can be calculated: C_{Li}/GFR, which represents the fractional excretion of fluid from the proximal tubule; $GFR - C_{Li}$, which is a measure of the absolute amount of fluid reabsorbed from the proximal tubules; UV/C_{Li}, which estimates the fractional excretion of water from the tubular regions beyond the proximal tubule and C_{Na}/C_{Li}, which is the fractional excretion of sodium from these tubular areas; $C_{Li} - UV$ represents the absolute amount of water reabsorbed after the proximal tubule and $C_{Li} - C_{Na}$ is the absolute amount of sodium reabsorbed by these tubular regions.

Statistics

The absolute and percentage changes given in the text represent the mean of individual changes recorded in each animal. Mean values ± s.e. mean are used. Statistical analysis was by paired Student's t test within groups and the unpaired Student's t test between groups. Differences were taken to be statistically significant at the 5% level.

Results

The data from the time control studies are included in Table 1 and 2 and show that neither blood pressure nor any of the renal haemodynamic or excretory variables changed in response to an injection of saline at the mid-point of the experiment.

Administration of the low dose of amlodipine (Table 1) to normotensive rats significantly reduced blood pressure by 12 ± 3 mmHg ($P < 0.01$) but had no effect on either renal blood flow or glomerular filtration rate. This dose of amlodipine caused significant increases in urine flow, of 70% ($P < 0.01$), absolute sodium excretion, of 91% ($P < 0.01$), and fractional sodium excretion, of 113% ($P < 0.05$), but did not change fractional lithium excretion. The pattern of tubular reabsorption responses to the low dose of amlodipine in the normotensive rats (Table 2) showed that while there was no significant change

Table 2 Effect of amlodipine on tubular reabsorptive function in normotensive rats

	Vehicle ($n = 7$)		*Amlodipine* ($200\,\mu g\,kg^{-1}$) ($n = 7$)		*Amlodipine* ($400\,\mu g\,kg^{-1}$) ($n = 8$)	
	Before	*After*	*Before*	*After*	*Before*	*After*
$GFR - C_{Li}$ ($\mu l\,min^{-1}\,kg^{-1}$)	2452 ± 357	2612 ± 344	3529 ± 436	2848 ± 538	3393 ± 390	3085 ± 243
UV/C_{Li} (%)	13.4 ± 7.1	6.5 ± 1.0	9.5 ± 2.7	11.7 ± 2.8	7.0 ± 1.0	10.2 ± 0.6**
C_{Na}/C_{Li} (%)	11.1 ± 2.2	11.3 ± 2.5	13.4 ± 4.0	16.7 ± 4.4**	11.6 ± 1.8	19.8 ± 2.1***
$CL_i - UV$, ($\mu l\,min^{-1}\,kg^{-1}$)	777 ± 149	765 ± 138	707 ± 121	854 ± 190	792 ± 80	978 ± 86**
$C_{Li} - C_{Na}$ ($\mu l\,min^{-1}\,kg^{-1}$)	736 ± 125	735 ± 112	666 ± 110	793 ± 171	712 ± 75	863 ± 80**

The P values represent a comparison between a mean of the two clearances before and the two clearances following drug or vehicle administration. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. $GFR - C_{Li}$ = absolute reabsorption at the proximal tubule; UV/C_{Li} = fractional excretion of water from the distal nephron regions; C_{Na}/C_{Li} = fractional excretion of sodium from the distal nephron regions; $C_{Li} - UV$ = absolute reabsorption of water from the distal nephron regions; $C_{Li} - C_{Na}$ = absolute reabsorption of sodium from the distal nephron regions.

in the absolute amount of fluid reabsorbed at the proximal tubule ($GFR - C_{Li}$) or in the fractional excretion of water (UV/C_{Li}) by the nephron regions after the proximal tubule, there was a significant increase ($P < 0.01$) in fractional sodium excretion, of 88%, beyond the proximal tubule in spite of absolute reabsorption of water ($C_{Li} - UV$) and sodium ($C_{Li} - C_{Na}$) remaining unchanged.

Infusion of the high dose of amlodipine into the normotensive animals (Table 1) reduced blood pressure significantly ($P < 0.05$), by 12 ± 4 mmHg, and did not alter either renal blood flow or glomerular filtration rate. However, there were significant increases in urine flow of 105% ($P < 0.001$), absolute sodium excretion, of 145% ($P < 0.001$), fractional sodium excretion, of 142% ($P < 0.001$), and fractional lithium excretion, of 29% ($P < 0.001$). The tubular reabsorptive changes in the normotensive animals (Table 2) showed that the high dose of amlodipine had no effect on the absolute amount of fluid reabsorbed from the proximal tubule ($GF - C_{Li}$) but that there were significant increases in the fractional excretion in both water (UV/C_{Li}) and sodium (C_{Na}/C_{Li}), of 55% ($P < 0.01$) and 88% ($P < 0.001$), respectively, by the nephron regions following the proximal tubule. At the same time there were significant increases in the absolute reabsorption of water ($C_{Li} - UV$) and sodium ($C_{Li} - C_{Na}$), by 26% ($P < 0.01$) and 18% ($P < 0.01$), respectively, in the tubular regions after the proximal tubule. The magnitude of the absolute changes in the tubular variables recorded in response to the high dose of amlodipine were slightly but not significantly, greater than those obtained with the low dose of drug.

Three groups of hypertensive rats were studied and the mean blood pressure of all these animals in the period before drug or vehicle administration was 161 ± 4 mmHg which was significantly ($P < 0.001$) higher than the 138 ± 4 mmHg in the normotensive animals, although mean renal blood flow and glo-

Table 3 Effect of amlodipine on blood pressure and renal function in hypertensive rats

	Vehicle ($n = 8$)		*Amlodipine* ($200\,\mu g\,kg^{-1}$) ($n = 7$)		*Amlodipine* ($400\,\mu g\,kg^{-1}$) ($n = 7$)	
	Before	*After*	*Before*	*After*	*Before*	*After*
BP (mmHg)	162 ± 5	157 ± 6	161 ± 5	151 ± 8	164 ± 8	137 ± 9**
RBF ($ml\,min^{-1}\,kg^{-1}$)	16.2 ± 1.3	16.1 ± 1.1	20.5 ± 1.8	21.7 ± 2.0	20.6 ± 2.0	22.8 ± 1.6
GFR ($ml\,min^{-1}\,kg^{-1}$)	4.49 ± 0.29	4.41 ± 0.32	3.61 ± 0.46	3.54 ± 0.39	3.85 ± 0.28	4.86 ± 0.56*
UV ($\mu l\,min^{-1}\,kg^{-1}$)	31.7 ± 4.2	34.2 ± 4.1	49.2 ± 7.6	76.6 ± 14.1*	38.2 ± 5.6	114.7 ± 18.2**
$U_{Na}V$ ($\mu mol\,min^{-1}\,kg^{-1}$)	5.2 ± 0.9	6.1 ± 1.0	7.3 ± 1.4	14.0 ± 3.4*	6.4 ± 1.4	24.4 ± 4.5**
Fe_{Na} (%)	0.86 ± 0.14	1.06 ± 0.17	1.50 ± 0.29	2.80 ± 0.50**	1.20 ± 0.25	3.58 ± 0.34***
FE_{Li} (%)	16.58 ± 0.69	18.18 ± 0.92	23.7 ± 1.9	29.7 ± 2.0*	19.7 ± 1.5	26.7 ± 2.2**

The P values represent a comparison between a mean of the two clearances before and the two following administration of the drug or vehicle. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.01$. Abbreviations as in Table 1.

Table 4 Effect of amlodipine on tubular reabsorptive function in hypertensive rats

	Vehicle		*Amlodipine* (200 $\mu g\,kg^{-1}$)		*Amlodipine* (400 $\mu g\,kg^{-1}$)	
	Before	*After*	*Before*	*After*	*Before*	*After*
$GFR-C_{Li}$ ($\mu l\,min^{-1}\,kg^{-1}$)	3360 ± 473	3097 ± 434	2767 ± 362	2492 ± 291	2565 ± 412	3593 ± 468
UV/C_{Li} (%)	4.18 ± 0.37	4.40 ± 0.60	6.99 ± 1.80	8.28 ± 1.53	5.26 ± 1.53	9.13 ± 1.94**
C_{Na}/C_{Li} (%)	5.07 ± 0.72	6.59 ± 1.29	6.47 ± 1.25	9.45 ± 1.63*	5.88 ± 1.11	13.65 ± 1.61**
$C_{Li}-UV$ ($\mu l\,min^{-1}\,kg^{-1}$)	722 ± 73	777 ± 89	790 ± 116	967 ± 128**	701 ± 27	1149 ± 99**
$C_{Li}-C_{Na}$ ($\mu l\,min^{-1}\,kg^{-1}$)	713 ± 76	757 ± 87	787 ± 108	945 ± 117**	697 ± 29	1131 ± 93**

The *P* values represent a comparison between the mean of the two clearance periods before and the two clearance periods following administration of the drug or vehicle. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Abbreviations as in Table 2.

merular filtration rate were the same in both normotensive and hypertensive animals at the start of the experiment. In comparison to the normotensive rats, the hypertensive animals had significantly smaller urine flow rates (39.8 ± 3.7 versus 61.8 ± 8.4 $\mu l\,min^{-1}\,kg^{-1}$; $P < 0.05$), absolute sodium excretion (6.3 ± 0.7 versus 15.0 ± 2.4 $\mu mol\ min^{-1}\ kg^{-1}$; $P < 0.01$) and fractional sodium excretion (1.18 ± 0.14 versus 2.70 ± 0.39%; $P < 0.01$). These differences were also reflected at the tubular level as fractional excretion of sodium in these late nephron regions (C_{Na}/C_{Li}) was 5.90 ± 6.4% in the hypertensive rats which was significantly less ($P < 0.01$) than 12.0 ± 1.5% in the normotensive rats.

The data from the time control hypertensive rats showed that there were no consistent changes over the course of the experiment in either blood pressure, renal haemodynamics or excretory function. This situation was comparable to that found in the normotensive time control animals. Administration of amlodipine at low doses to hypertensive animals (Table 3) caused a small non-significant depression of blood pressure, had no effect on renal blood flow and glomerular filtration rate but did cause significant increases in urine flow, of 65% ($P < 0.05$), absolute sodium excretion, of 91% ($P < 0.05$), and fractional sodium excretion, of 96% ($P < 0.01$) and fractional lithium excretion, of 28% ($P < 0.05$). Table 4 shows that the low dose of amlodipine had no effect on the absolute reabsorption of fluid from the proximal tubule ($GFR-C_{Li}$) but beyond this tubular segment, although there was no change in the fractional excretion of water (UV/GFR), there were significant increases in sodium (C_{Na}/C_{Li}), of 51% ($P < 0.05$) absolute reabsorption of water ($C_{Li} - UV$), of 24% ($P < 0.01$) and sodium ($C_{Li} - C_{Na}$), of 22% ($P < 0.01$).

The high dose of amlodipine given to the hypertensive animals (Table 3) caused a significant reduction in blood pressure, of 27 ± 5 mmHg, which was significantly greater than that observed in the hypertensive rats given the low dose of drug ($P < 0.01$) and the normotensive animals given either low or high doses of amlodipine (both $P < 0.05$). In this group of hypertensive rats, renal blood flow did not change but there were significant increases in glomerular filtration rate, of 25% ($P < 0.05$), urine flow, of 224% ($P < 0.01$), absolute sodium excretion, of 421% ($P < 0.01$), fractional sodium excretion, of 259% ($P < 0.001$) and a smaller rise in fractional lithium clearance, of 38% ($P < 0.01$). The pattern and magnitude of these excretory responses was similar to that obtained in the hypertensive rats given the low dose of amlodipine and the normotensive rats given both doses of drug.

The tubular reabsorption responses to the high dose of amlodipine in the hypertensive rats (Table 4) show that although the absolute reabsorption of fluid in the proximal tubules ($GFR-C_{Li}$) did not change, beyond this point there were significant increases in fractional excretion of both water (UV/C_{Li}) and sodium (C_{Na}/C_{Li}), of 84% ($P < 0.01$) and 121% ($P < 0.01$), respectively, as well as significant increases in the absolute reabsorption of water ($C_{Li} - UV$) and sodium ($C_{Li} - C_{Na}$), of 63% ($P < 0.01$) and 60% ($P < 0.01$), respectively. The increases in fractional water (UV/C_{Li}) and sodium excretion (C_{Na}/C_{Li}) beyond the proximal tubule in response to the high dose of amlodipine were similar to those recorded when the low dose was given to the hypertensive animals. However, in the hypertensive rats the increases in absolute reabsorption of water ($C_{Li} - UV$) and sodium ($CL_i - C_{Na}$) by the late tubular regions during the high dose amlodipine was significantly greater than that found in response to the low dose of drug ($P < 0.05$ and $P < 0.01$, respectively) and in those found in response to the high ($P < 0.02$ and $P < 0.01$, respectively) and low

doses of amlodipine (both $P < 0.05$) in the normotensive rats.

Discussion

A comparison was undertaken of the renal haemodynamic and tubular responses to calcium channel blockade in normotensive and hypertensive rats using the recently developed dihydropyridine derivative amlodipine (Burges *et al.*, 1987). The blood pressure, renal haemodynamic and excretory variables of the normotensive rats were very similar to those previously reported (Johns, 1985) under these experimental conditions. Blood pressure in the hypertensive animals was significantly higher than in the normotensive rats and although renal blood flow and glomerular filtration rate were similar, urine flow and sodium excretion were approxiamtely 40% less in the hypertensive rats. The reasons for the decreased fluid output are unclear but they may reflect inherent differences in tubular function between the two types of animal. Amlodipine adminstration decreased blood pressure to a similar extent in normotensive rats at both doses, whereas in the hypertensive animals the blood pressure reduction did not achieve statistical significance at the low dose and at the higher dose the fall was larger than in the normotensive animals. Such a vasodepressor effect has been observed with most of the calcium channel blocking drugs and probably reflects on action at vascular smooth muscle (Godfraind *et al.*, 1986).

Renal blood flow was unchanged by either dose of amlodipine in normotensive or hypertensive rats which was similar to our previous observations with nifedipine, diltiazem and nitrendipine (Johns, 1985; Johns & Manitius, 1986) and is consistent with other reports (Loutzenhiser & Epstein, 1985). Amlodipine also had no effect on glomerular filtration rate in normotensive or hypertensive animals receiving the low dose of drug. However, in hypertensive rats given the high dose of amlodipine there was a rise in glomerular filtration rate similar to that observed in hypertensive man (Reams *et al.*, 1987). The possibility exists that within the kidneys of these hypertensive rats the fall in perfusion pressure together with an action of the drug on the renal vasculature itself, had a greater effect at the afferent than the efferent arterioles which would cause an increase in filtration pressure and hence filtration rate.

There was an approximate doubling of urine flow and sodium excretion in the normotensive rats receiving the low dose of amlodipine which was only slightly greater when the high dose was given. The magnitude of the natriuretic and diuretic responses did not appear to be dose-related suggesting that the action of amlodipine was being limited by some other mechanism. These excretory responses to amlodipine are comparable to those reported for other calcium channel blocking drugs such as diltiazem, verapamil, nifedipine and nitrendipine (Brown & Churchill, 1983; Dietz *et al.*, 1983; Johns, 1985; Johns & Manitius, 1986).

Amlodipine also caused a natriuresis and diuresis in the hypertensive rats and, in spite of a doubling of the dose, the size of the increased sodium and water output was not significantly different; however, at the high dose of amlodipine there was a large reduction in blood pressure, of 27 mmHg, which would exert an important antinatriuretic and antidiuretic effect (Roman & Cowley, 1985). These findings are similar to those of DiBona (1985) and Nordlander *et al.* (1985) who found that the diuresis and natriuresis caused by felodipine in spontaneously hypertensive rats persisted in the face of reductions in blood pressure.

Thomsen (1984) has developed the technique of lithium clearance and has validated the concept that under most normal conditions lithium ions are reabsorbed at the proximal tubule in the same manner as sodium ions but that they pass through the loop of Henlé, distal convoluted tubule and collecting duct without undergoing further reabsorption. Consequently, calculations can be carried out to determine the pattern of water and sodium reabsorption in the proximal tubule separate from the later nephron segments.

The high dose of amlodipine given to normotensive rats approximately doubled water and sodium output without changing absolute reabsorption of fluid from the proximal tubules. Thus, the fluid load presented to the loop of Henlé and subsequent nephron segments was constant and therefore the natriuresis and diuresis must have resulted in differential reabsorptive adjustments in these regions of the nephron. The fractional excretion of water and sodium beyond the proximal tubule were increased yet this occurred in the face of increased absolute reabsorption of fluid. One explanation of these findings could be that there was depressed reabsorption in the loop of Henlé which would increase fluid emerging into the distal convoluted tubule. This might be sufficient to initiate a compensatory increase in absolute sodium and water reabsorption in the distal convoluted tubule (Seldin & Giebisch, 1985) which was large enough to influence the overall measure of fluid reabsorption by this technique. It would seem that compensation was not complete as the fraction of fluid excreted by these tubular segments was increased to such a degree that the natriuresis and diuresis still occurred. The pattern of tubular responses in the hypertensive animals given the high dose of amlodipine was

similar and the underlying mechanisms were probably analagous although the situation was complicated in this group of animals because of the concomitant increased glomerular filtration rate. There was an indication, however, that the high dose of amlodipine caused larger changes in tubular function in the hypertensive rats than in the normotensive animals.

The pattern of excretory responses in the hypertensive rats given the low dose of amlodipine closely followed that observed with the high dose of drug and the underlying mechanisms are probably very similar. In the normotensive rats given the low dose of amlodipine there were some qualitative differences. At this low dose amlodipine substantially increased sodium and water output. Absolute reabsorption of fluid in the proximal tubule and in the nephron regions beyond the proximal tubule remained unchanged but there was an increased fractional excretion of sodium in these regions. One suggestion is that amlodipine caused an increased load to be presented to the nephron segments after the proximal tubule which was not sufficient to activate a compensatory mechanism to increase absolute fluid reabsorption, but was able to cause a natriuresis and diuresis. Again, a possible explanation would be of reduced fluid reabsorption along the loop of Henlé resulting in increased fluid load being presented to the nephron segments beyond the loop of Henlé.

In summary, the data show that administration of amlodipine, at $200\,\mu g\,kg^{-1}$ plus $50\,\mu g\,kg^{-1}\,h^{-1}$ decreased blood pressure in the normotensive rats, although this fall did not reach statistical significance in the hypertensive animals, and $400\,\mu g\,kg^{-1}$ plus $100\,\mu g\,kg^{-1}\,h^{-1}$, decreased blood pressure in both groups of rats. Renal blood flow was not changed at either dose in normotensive or hypertensive rats and glomerular filtration rate was increased only at the high dose given to the hypertensive animals. Both doses of amlodipine caused a natriuresis and diuresis, of approximately similar magnitude, in normotensive and hypertensive rats which occurred without change in proximal tubular reabsorption. The associated changes in tubular function were consistent with a possible site of action at the loop of Henlé together with more distal segments of the nephron. Whether this action of amlodipine is caused by an inhibition of the transporting mechanisms within the cells of the loop of Henlé, distal convoluted tubule and collecting ducts or due to a vascular change which might result in an increase in blood flow to the deeper regions of the kidney remains to be determined.

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