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A study of the action of amlodipine on adrenergically regulated sodium handling by the kidney in normotensive and hypertensive rats

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1 An investigation was undertaken to examine the effect of calcium channel blockade, induced by amlodipine, on the ability of the renal sympathetic nerves to cause an antidiuresis and antinatriuresis in normotensive Sprague Dawley and spontaneously hypertensive rats anaesthetized with pentobarbitone.

2 Low frequency renal nerve stimulation in normotensive rats, which did not change renal blood flow, caused a 15% reduction in glomerular filtration rate and was associated with falls in urine flow of 37%, absolute sodium excretion of 47%, and fractional sodium excretion of 38%. The magnitude of these renal excretory changes was unaffected by prior administration of amlodipine at either $200\,\mu g\,kg^{-1}$ plus $50\,\mu g\,kg^{-1}\,h^{-1}$ or $400\,\mu g\,kg^{-1}$ plus $100\,\mu g\,kg^{-1}\,h^{-1}$. Amlodipine given in the higher dose, decreased basal levels of blood pressure and increased basal urine flow and sodium excretion.

3 In spontaneously hypertensive rats, renal nerve stimulation minimally affected renal haemodynamics but decreased urine flow, absolute and fractional sodium excretion by 29%, 31% and 24%, respectively.

4 Similar renal nerve stimulation in spontaneously hypertensive rats given amlodipine at $200\,\mu g\,kg^{-1}$ plus $50\,\mu g\,kg^{-1}\,h^{-1}$ or $400\,\mu g\,kg^{-1}$ plus $100\,\mu g\,kg^{-1}\,h^{-1}$ caused minimal changes in renal haemodynamics and in the excretion of water and sodium. The higher dose of drug resulted in decreased blood pressure and increased basal rates of urine flow and sodium excretion.

5 These data show that in spontaneously hypertensive rats but not normotensive rats, calcium channel blockade inhibited the ability of the renal nerves to stimulate the reabsorptive processes for sodium at the renal tubule. This indicated that in spontaneous hypertension the post-receptor mechanisms had changed and become more dependent on the inward movement of calcium.

Introduction

The sympathetic innervation of the kidney extends to all components of the renal vasculature (Barajas, 1978) and tubules (Barajas *et al.*, 1984). In functional terms there is now good evidence to show that when the renal nerves are stimulated at low levels that have no influence on renal haemodynamics, there is an increased renin secretion and a decreased excretion of sodium and water. These effects have been taken to represent a direct action of the nerves on the renin-containing cells of the afferent arteriole to cause renin release, and on the epithelial cells of the proximal tubule and the thick limb of the ascending loop of Henlé (DiBona, 1982; DiBona & Sawin, 1982) to stimulate the sodium transporting processes.

At the renal tubular level, the ability of the renal nerves to increase sodium reabsorption has been shown to involve activation of α-adrenoceptors of the α_1-subtype in the dog (Osborn *et al.*, 1983), rabbit (Hesse & Johns, 1984) and rat (Johns & Manitius, 1986a). Radioligand binding analysis of renal cortical tissue showed that α_2-adrenoceptors are far more abundant than α_1-adrenoceptors, approximately 3 : 1 (Sanchez *et al.*, 1986) but their functional role is unclear. Nevertheless, during increased dietary sodium intake and in genetic models of experimental hypertension there is an increase in the density of α-adrenoceptors that is relatively greater for the α_2-subtype (Pettinger *et al.*, 1982; Graham *et al.*, 1982). A possible consequence

of these changes in hypertension is that α_2-adrenoceptors might take over the functions of the α_1-adrenoceptors at the tubular level.

Adrenergic transmission at vascular smooth muscle is sensitive to blockade by the calcium channel blocking drugs through their ability to block inward movement of calcium into the effector cells (Godfraind *et al.*, 1986). Studies have been undertaken to define whether one or both α-adrenoceptor subtypes can be blocked by this class of compound but the situation is not clear. Van Zwieten and coworkers (1985) contend that α_2-adrenoceptor- mediated responses are primarily inhibited by the calcium channel blocking drugs, while Vanhoutte & Rimele (1982) have proposed that their inhibition of effector responses is dependent on the degree of functional specialization of the neuro-effector junction at particular tissues. As regards neuro-transmission at the renal nerve-epithelial cell junctions of the renal tubule, evidence to date has shown that the calcium channel blocking drugs have no action at this site (Herod & Johns, 1985; Johns & Manitius, 1986b).

The possibility arises that because of the changes in α_2-adrenoceptor density which occur in the renal cortex of hypertensive models, the adrenergically induced responses could become susceptible to inhibition by the calcium channel blocking drugs. This was addressed in the present study by electrically stimulating the renal nerves at low frequencies, such that renal haemodynamics were minimally altered and comparing the magnitude of the changes in sodium and water output in both normotensive and spontaneously hypertensive rats in the absence and presence of the calcium channel blocking drug, amlodipine (Burges *et al.*, 1987).

Methods

Normotensive male albino Sprague-Dawley rats (325–425 g) were obtained from the Departmental Animal House. Spontaneously hypertensive rats (Kyoto) were purchased from O1ac (Bicester) at 8 weeks of age and were maintained in the Animal House until use, between two and three months later (315–400 g). The rats were anaesthetized with sodium pentobarbitone, 60 mg kg^{-1}, i.p., followed by a continuous intravenous infusion at 15 mg kg^{-1} h^{-1}. A cannula was inserted into the right carotid artery for the measurement of blood pressure (Statham P23ID transducer attached to a Grass model 7D polygraph) and removal of blood samples. A cannula was placed in the left jugular vein and an infusion begun, at 6 ml h^{-1}, of a 150 mM NaCl solution which was continued until the end of the experiment.

A ventral mid-line incision was used to expose the left kidney, its ureter was cannulated and the artery cleared so that an electromagnetic flow probe (Carolina EP100 series) could be fitted to allow renal blood flow to be measured directly (Carolina FM501 flowmeter linked to the Grass polygraph). A Zeiss model 212 surgical microscope was used to identify the renal nerves traversing to the kidney; they were dissected for a short length to enable them to be placed in bipolar silver wire stimulating electrodes and were then sectioned. The distal ends of the renal nerves were stimulated for a 5–10 s period at 10 Hz (15 V, 0.2 ms) which caused a blanching of the kidney and thereby established the functionality of the nerves.

Renal function measurements

On completion of surgery the animals received 2 ml of saline containing inulin (10 mg ml^{-1}) and the infusate was changed to saline and inulin (10 mg ml^{-1}) which was given until the end of the experiment. The animals were allowed 2.5 h to stabilise before the experiments were begun.

The experiments consisted of a sequence of five 15 min clearance periods, two before, one during and two following a period during which the renal nerves underwent electrical stimulation. The nerves were stimulated for 20 min at rates which were just below threshold for causing a measurable reduction in renal blood flow and this required frequencies of between 0.8 to 2 Hz at 15 V, 0.2 ms duration. Urine was not collected for the 5 min after the start or end of renal nerve stimulation in order to allow preformed urine to clear from the ureteral cannula.

Blood samples (0.6 ml) were taken from the carotid artery at the beginning of clearance period 1 and end of clearance periods 2, 3 and 5, and immediately centrifuged. The plasma was separated and placed in the deep freeze while the red cells were resuspended in an equal volume of saline and reinfused into the animal as quickly as possible. Mean blood pressure and renal blood flow were averaged over each clearance period using a BBC microcomputer and Unilab interface linked to a Torch Z80 disc drive which accepted input data from the polygraph (Emmerson & Johns, 1986).

Inulin in plasma and urine samples was estimated as previously described (Johns *et al.*, 1976) and the clearance of inulin was taken as a measure of glomerular filtration rate. Plasma and urinary sodium concentrations were measured by emission spectroscopy (Corning 410C).

Experimental protocols

Six groups of rats were studied, 3 of which were normotensive and 3 spontaneously hypertensive. One group of each type was subjected to one of the fol-

lowing procedures: (a) time control: a bolus of saline (0.1 ml $100\,g^{-1}$ body wt, i.v.) was given and the infusion continued; (b) low dose amlodipine: a bolus of amlodipine, $200\,\mu g\,kg^{-1}$ i.v. (in 0.1 ml $100\,g^{-1}$ body weight) was given and the infusate changed to one containing amlodipine at a concentration such that it was delivered at $50\,\mu g\,kg^{-1}\,h^{-1}$; (c) high dose amlodipine: $400\,\mu g\,kg^{-1}$ amlodipine i.v., was administered in 0.1 ml $100\,g^{-1}$ body weight and the infusion changed to one containing amlodipine at a concentration such that the animal received $100\,\mu g\,kg^{-1}\,h^{-1}$.

Infusion of vehicle or drug started 20 min before the sequence of clearance periods was begun.

Drug

The dihydropyridine derivative, amlodipine (3-ethyl,5-methyl, 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl,3,5-pyridine carboxylate) was supplied by Pfizer, Kent. It was dissolved in saline at $500\,\mu g\,ml^{-1}$ and this stock solution was kept refrigerated and used within three days. Bolus injections were made by diluting the stock in saline. The amlodipine for infusion was diluted with the saline containing inulin and pentobarbitone.

Statistics

In the text, the absolute and percentage changes quoted represent the mean value of the individual changes recorded for each animal. The renal responses to nerve stimulation were measured by taking an average of values of the two clearances before and the two following stimulation and comparing it with that value recorded during the stimulation period. All data are expressed as means $\pm$ s.e. mean. Student's paired t test was used for intra-group comparisons and Student's unpaired t test for inter-group analysis. Differences between means were considered significant at the 5% level.

Results

The results of low frequency renal nerve stimulation in the normotensive rats are shown in Table 1. In the time control group, blood pressure was well maintained throughout each experiment. During the period of renal nerve stimulation renal blood flow fell slightly, but did not reach statistical significance, while there were significant reductions in: glomerular filtration rate of 15% ($P < 0.05$); urine flow of 37% ($P < 0.01$); absolute sodium excretion of 47% ($P < 0.01$); and fractional sodium excretion of 38% ($P < 0.05$).

The animals infused with the low dose of amlodipine had a basal blood pressure lower than that of the time control group, whereas renal blood flow, glomerular filtration rate, urine flow, absolute and fractional sodium excretions were higher than the time control group although these differences were not statistically significant. Basal blood pressure remained stable over the course of the observations. Stimulation of the nerves at low frequencies caused minimal falls in renal blood flow and glomerular filtration rate but was associated with significant reductions in: urine flow of 29% ($P < 0.05$); absolute sodium excretion of 37% ($P < 0.01$); and fractional sodium excretion of 31% ($P < 0.01$). The renal excretory responses to nerve stimulation in the animals given the low dose of amlodipine were not statistically different in size from those obtained in the time control group.

Administration of the high dose of amlodipine resulted in a group of rats which had a basal blood pressure significantly ($P < 0.01$) less than the time control group and had increased basal levels of urine flow ($P < 0.01$), absolute ($P < 0.05$) and fractional ($P < 0.01$) sodium excretions. Blood pressure in these animals remained stable over the period of measurement and while renal nerve stimulation caused minor changes in renal blood flow and glomerular filtration rate, there were significant reductions in urine flow of 37% ($P < 0.05$), absolute sodium excretion of 48% ($P < 0.01$), and fractional sodium excretion of 44% ($P < 0.001$). The magnitude of the absolute and fractional sodium excretory responses to renal nerve stimulation in the animals given the high dose of amlodipine were significantly greater than those recorded in the time control animals (both $P < 0.05$), although the percentage changes were identical, but could not be distinguished statistically from those obtained in the group of animals receiving the low dose of amlodipine.

Table 2 presents the effects of low frequency stimulation on renal function in the spontaneously hypertensive rats in the absence and presence of amlodipine. In the time control rats, blood pressure did not change over the course of the experiment. Stimulation of the renal nerves had minimal effects on renal blood flow and glomerular filtration rate but significantly decreased urine flow by 23% ($P < 0.01$), absolute sodium excretion by 31% ($P < 0.05$) and fractional sodium excretion by 24% ($P < 0.01$).

Basal blood pressure in the animals infused with the low dose of amlodipine was not significantly different from the time control hypertensive rats and even though there was a gradual fall over the course of the experiment this did not become statistically significant. The basal levels of renal haemodynamic and excretory variables were higher than in the time

Table 1 Effect of low-frequency renal nerve stimulation on blood pressure and renal function in normotensive rats

	Saline infusion ($n = 8$)			*Amlodipine* ($200\,\mu g\,kg^{-1}$ + $50\,\mu g\,kg^{-1}\,h^{-1}$) ($n = 7$)			*Amlodipine* ($400\,\mu g\,kg^{-1}$ + $100\,\mu g\,kg^{-1}\,h^{-1}$) ($n = 7$)		
	Basal	*Stim.*	*Recovery*	*Basal*	*Stim.*	*Recovery*	*Basal*	*Stim.*	*Recovery*
Blood pressure (mmHg)	137 ± 4	133 ± 5	128 ± 7	119 ± 9	114 ± 10	111 ± 11	110 ± 4	106 ± 5	104 ± 5
Renal blood flow, ($ml\,min^{-1}\,kg^{-1}$)	12.5 ± 2.0	11.4 ± 1.3	12.4 ± 1.4	14.2 ± 1.6	14.0 ± 1.6	13.6 ± 1.5	13.0 ± 0.8	13.7 ± 1.1	15.9 ± 1.2
Glomerular filtration rate ($ml\,min^{-1}\,kg^{-1}$)	2.69 ± 0.40	2.21* ± 0.37	2.54 ± 0.42	2.99 ± 0.36	2.76 ± 0.37	3.25 ± 0.31	3.46 ± 0.33	3.00 ± 0.36	3.07 ± 0.23
Urine flow, ($\mu l\,min^{-1}\,kg^{-1}$)	30.7 ± 4.8	20.9** ± 4.0	34.4 ± 6.1	47.1 ± 10.2	34.1* ± 6.4	54.1 ± 9.3	73.7 ± 9.9	43.9* ± 7.4	74.0 ± 13.9
Absolute sodium excretion ($\mu mol\,min^{-1}\,kg^{-1}$)	6.23 ± 1.50	3.80** ± 1.18	6.88 ± 1.90	10.47 ± 2.78	7.03** ± 1.76	12.51 ± 2.75	17.06 ± 3.56	8.75** ± 2.62	17.25 ± 4.44
Fractional sodium excretion (%)	1.54 ± 0.28	1.07* ± 0.24	1.95 ± 0.54	2.70 ± 0.85	1.95** ± 0.63	2.82 ± 0.78	3.84 ± 0.55	2.02*** ± 0.39	3.40 ± 0.51

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

Table 2 Effect of low-frequency renal nerve stimulation on blood pressure and renal function in spontaneously hypertensive rats

	Saline infusion ($n = 7$)			*Amlodipine* ($200\,\mu g\,kg^{-1}$ + $50\,\mu g\,kg^{-1}\,h^{-1}$) ($n = 8$)			*Amlodipine* ($400\,\mu g\,kg^{-1}$ + $100\,\mu g\,kg^{-1}\,h^{-1}$) ($n = 7$)		
	Basal	*Stim.*	*Recovery*	*Basal*	*Stim.*	*Recovery*	*Basal*	*Stim.*	*Recovery*
Blood pressure (mmHg)	148 ± 6	150 ± 7	145 ± 9	150 ± 8	138 ± 8	127 ± 8	133 ± 8	119 ± 6	108 ± 7
Renal blood flow, ($ml\,min^{-1}\,kg^{-1}$)	16.7 ± 1.8	16.0 ± 1.7	16.3 ± 1.8	23.3 ± 1.5	21.3 ± 1.9	20.7 ± 1.9	20.8 ± 1.6	18.7 ± 1.3	19.4 ± 1.7
Glomerular filtration rate ($ml\,min^{-1}\,kg^{-1}$)	4.28 ± 0.40	3.65 ± 0.41	3.75 ± 0.37	3.53 ± 0.51	2.69* ± 0.40	2.95 ± 0.45	4.29 ± 0.35	3.76 ± 0.49	3.69 ± 0.46
Urine flow, ($\mu l\,min^{-1}\,kg^{-1}$)	49.4 ± 4.7	33.0** ± 3.5	38.2 ± 3.4	73.4 ± 16.6	53.5 ± 8.3	56.7 ± 7.9	99.7 ± 18.1	92.0 ± 19.6	98.4 ± 12.6
Absolute sodium excretion, ($\mu mol\,min^{-1}\,kg^{-1}$)	8.40 ± 1.37	4.94* ± 0.58	6.26 ± 0.98	14.17 ± 4.26	9.78 ± 2.25	10.84 ± 2.04	17.95 ± 3.18	16.49 ± 3.04	18.55 ± 2.28
Fractional sodium excretion (%)	1.49 ± 0.23	1.02** ± 0.11	1.25 ± 0.17	2.79 ± 0.62	2.77 ± 0.57	2.71 ± 0.43	3.22 ± 0.48	3.65 ± 0.75	4.55 ± 0.96

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

control rats but the differences did not reach statistical significance. Renal nerve stimulation in animals infused with the low dose of amlodipine had a minimal effect on renal blood flow and caused a significant reduction in glomerular filtration rate of 17% ($P < 0.05$). Although there were small reductions in urine flow, absolute and fractional sodium excretions from basal levels during renal nerve stimulation, these changes did not reach statistical significance and the variables generally remained at these lower levels during the recovery period so that overall there were no meaningful changes. The relatively blunted responses in sodium output to nerve stimulation were very different in pattern from those observed in the hypertensive time control animals. A comparison of the magnitude of the absolute changes in the excretory responses in the normotensive and hypertensive groups given the low dose of amlodipine showed that although the reduction in urine flow and absolute sodium excretion could not be distinguished from those in the hypertensive animals, the fall in fractional sodium excretion was significantly ($P < 0.05$) less in the hypertensive rats.

Blood pressure in the hypertensive animals infused with the high dose of amlodipine was significantly lower than that observed in the hypertensive time control group ($P < 0.001$) and in the hypertensive group infused with the low dose of amlodipine ($P < 0.01$). Infusion of amlodipine in the higher dose was associated with a gradual decline in blood pressure by some 25 mmHg ($P < 0.05$). The basal values of renal haemodynamics in the animals infused with the high dose of amlodipine were the same as in the two other hypertensive groups. However, the basal levels of urine flow, absolute and fractional sodium excretions were significantly greater than those of the hypertensive time control group ($P < 0.05$, $P < 0.05$, $P < 0.01$, respectively) but not those of the hypertensive group infused with the low dose of amlodipine. Low frequency renal nerve stimulation caused minimal reductions in renal blood flow and glomerular filtration rate while the small reductions in urine flow, absolute and fractional sodium excretions were not statistically meaningful. The blunted responses to renal nerve stimulation in urine flow, absolute and fractional sodium excretion observed in the hypertensive animals given the high dose of amlodipine were again very different in pattern from those observed in the hypertensive time control animals but, because of the large differences in basal output of water and sodium, the magnitudes of the absolute changes were not significantly different. However, the reductions in absolute and fractional sodium excretion induced by renal nerve stimulation in hypertensive rats given high dose amlodipine were significantly ($P < 0.01$) less in magnitude than those

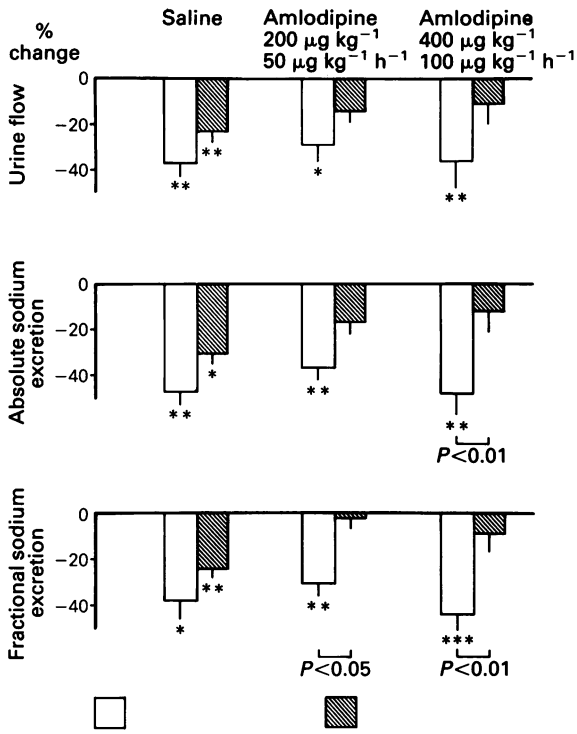

Figure 1 Comparison of the percentage changes in urine flow, absolute and fractional sodium excretions caused by stimulation of the renal nerves in normotensive (open columns) and spontaneously hypertensive (hatched columns) rats in the absence and presence of amlodipine. The P values represent Student's unpaired t test comparisons between normotensive and hypertensive groups while the asterisks represent Student's paired t test comparisons within each group of normotensive and hypertensive rats: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

observed in the counterpart normotensive rats. A comparison of the percentage changes in excretory variables to nerve stimulation in the absence and presence of amlodipine in the normotensive and spontaneously hypertensive rats is shown in Figure 1.

Discussion

The aim of this study was to determine whether the α-adrenoceptor-mediated process of neural stimulation of tubular sodium reabsorption was sensitive to inhibition by calcium channel blockade in spontaneously hypertensive and in normotensive rats. Calcium channel blockade was induced with amlodipine, a recently developed dihydropyridine com-

pound, which has been shown to be a potent blocker of calcium channels (Burges *et al.*, 1987). Preliminary studies were carried out to determine dose levels of amlodipine which gave either a small or moderate reduction in blood pressure. This criterion for calcium channel blockade was similar to that used in previous studies (Johns, 1985). The doses of amlodipine chosen for the present study were in the range of those reported previously (Burges *et al.*, 1985).

Amlodipine was administered at two dose levels, the lower of which had marginal effects on blood pressure in both normotensive and spontaneously hypertensive rats while at the higher dose of amlodipine blood pressure was markedly depressed in both groups of animals. These findings are similar to those previously described following amlodipine administration to spontaneously hypertensive rats (Johns, 1988) and were comparable to the blood pressure reductions reported when felodipine was given to this model of genetic hypertension (Nordlander *et al.*, 1985). This vasodepressor action of amlodipine was similar to that observed with other calcium channel blocking drugs and represented an action at vascular smooth muscle (Godfraind *et al.*, 1986). Amlodipine has been demonstrated to have a greater relative specificity for calcium channels in vascular smooth muscle than in cardiac tissue, a property shared by other dihydropyridines (Burges *et al.*, 1987). There was a tendency for blood pressure to fall progressively over the course of the experiment which was more apparent in the spontaneously hypertensive rats, particularly at the high dose level. This effect was possibly related to the fact that amlodipine has a relatively slow onset of action compared to other calcium channel blockers (Burges *et al.*, 1987), or it could be that the metabolic turnover of the drug was slower than the rate of infusion so that it became increasingly effective as the experiment progressed.

The lack of effect of either dose of amlodipine on renal haemodynamics in the normotensive rats is similar to observations made with many other calcium channel blockers (Loutzenhiser & Epstein, 1985). The present studies extend these observations to show that even in the spontaneously hypertensive rats the renal vasculature is minimally affected by calcium channel blockade. It was also evident that the basal level of both sodium and water output became greater as the infusion rate of amlodipine was increased which appeared to be a dose-related event in both the normotensive and spontaneously hypertensive rats. This natriuretic and diuretic action of calcium channel blocking drugs has been consistently reported in normotensive rats (Brown & Churchill, 1983; Johns, 1985; Johns & Manitius, 1986b) and was also described following felodipine administration to spontaneously hypertensive rats (Nordlander *et al.*, 1985). The mechanisms underlying this effect are not clear, as convincing evidence of an inhibition of tubular reabsorptive processes for sodium at specific sites along the nephron has not been produced (Johns, 1988).

The time control studies using the normotensive and spontaneously hypertensive rats demonstrated that the animals were stable over the period of measurement. Stimulation of the renal nerves at low frequencies had minimal effects on renal haemodynamics yet there were quite large reductions in both absolute and fractional sodium excretions which have been taken to reflect increased tubular reabsorption of sodium. These studies also showed that comparable renal nerve-induced tubular responses could be obtained in both groups of animals. It is now generally accepted that the changes in sodium output caused by these low rates of renal nerve stimulation reflect a direct action on the epithelial cell transporting processes in the proximal tubule (Pelayo *et al.*, 1983) and the ascending limb of the loop of Henlé (DiBona & Sawin, 1982; Bencsáth *et al.*, 1985).

Stimulation of the renal nerve in the normotensive rats at low rates in the presence of both the low and high doses of amlodipine had no effect on the magnitude of the antinatriuresis and antidiuresis compared to that obtained in the absence of the drug. The nerve-induced decreases in sodium and water output are mediated by α-adrenoceptors at the epithelial cells which have been shown to be of the α_1-subtype in the rat (Johns & Manitius, 1986a). The results of the present study clearly show that in these normotensive rats in which the calcium channels are blocked, as indicated by the fall in blood pressure, the α_1-adrenoceptors present on the epithelial cells do not require the movement of extracellular calcium into the cells in order to have their action. It is likely that the post-receptor mechanisms depend on the mobilisation of intracellular rather than extracellular calcium. These findings are similar to those previously reported (Herod & Johns, 1985; Johns & Manitius 1986b) in which diltiazem, nifedipine and nitrendipine were all incapable of blocking the ability of the renal nerves to cause an antinatriuresis and antidiuresis.

In the spontaneously hypertensive rats, amlodipine at both doses blunted the ability of the renal nerves to reduce both absolute and fractional sodium excretions. These findings are in marked contrast to those obtained in normotensive rats given amlodipine (as in the present study), diltiazem, nifedipine (Herod & Johns, 1985) or nitrendipine (Johns & Manitius, 1986b) which had no measurable effect on the renal nerve-induced antinatriuresis and antidiuresis. Two possibilities exist to explain this sensitivity of the tubular actions of the renal nerves

to calcium channel blockade in the spontaneously hypertensive rats. Firstly, the evidence of radioligand binding studies show that α_2-adrenoceptors, which are present in a ratio of 3:1 compared with α_1-adrenoceptors in the renal cortex (Sanchez *et al.*, 1986), are relatively increased in various forms of spontaneous hypertension (Graham *et al.*, 1982). It is therefore possible that these large numbers of α_2-adrenoceptors could take over functions normally subserved by α_1-adrenoceptors. They could, according to the proposition of van Zwieten and coworkers (1985), then become susceptible to blockade by the calcium channel blocking drugs. However, this particular hypothesis is unlikely as in a recent report DiBona & Sawin (1987) showed that, even in the spontaneously hypertensive rat, adrenergically mediated tubular sodium reabsorption depended on activation of α_1-adrenoceptors.

A second option is that in the spontaneously hypertensive rat, although α_1-adrenoceptors present on the epithelial cells still respond to neurally released noradrenaline, the cells are changed in some way so that the post-receptor intracellular events become dependent on the inward movement of calcium. Such a contention would be supported by the report of Kazda *et al.* (1985) who showed that α_1-adrenoceptor-mediated vasoconstriction in vascular smooth muscle of spontaneously hypertensive, but not normotensive, rats was blunted by calcium channel blockade. This observation together with the findings of the present study support the views of Vanhoutte & Rimele (1982) who proposed that the dependency of α-adrenoceptor-mediated processes on the inward movement of extracellular calcium was not related to the α-adrenoceptor subtype involved but was more related to the degree of specialisation of the neuro-effector junction.

The findings of the present study have shown that administration of amlodipine, at doses which have marginal or marked vasodepressor effects, to normotensive rats had no measurable effect on the ability of the renal nerves to stimulate tubular sodium reabsorption. Administration of the same doses of amlodipine to spontaneously hypertensive rats markedly attenuated the ability of the renal nerves to cause an antinatriuresis and antidiuresis. These results show that in normotensive rats the nerve-induced tubular responses, which are mediated by α_1-adrenoceptors on the epithelial cells, do not depend on extracellular calcium, but that in animals in which spontaneous hypertension is established there are changes within the epithelial cells indicating that the post-receptor events are altered in some way so that they become dependent on the inward movement of calcium ions in order to allow the cellular response to occur.

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