Br. J. Pharmacol. (1993), **108**, 948–958

Effects of high calcium diet on arterial smooth muscle function and electrolyte balance in mineralocorticoid-salt hypertensive rats

[1]Pertti Arvola, *Heikki Ruskoaho & [2]Ilkka Pörsti

Department of Biomedical Sciences, University of Tampere, P.O. Box 607, SF-33101 Tampere, Finland, and *Department of Pharmacology and Toxicology, University of Oulu, Kajaanintie 52 D, SF-90220 Oulu, Finland

1 The effects of a high calcium diet (2.5%) on blood pressure, electrolyte balance, plasma and tissue atrial natriuretic peptide (ANP), cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$), and arterial smooth muscle responses were studied in one-kidney deoxycorticosterone (DOC)-NaCl hypertensive Wistar rats.

2 Calcium supplementation for 8 weeks markedly attenuated the development of DOC-NaCl hypertension and the associated cardiac hypertrophy, and prevented the DOC-NaCl-induced sodium-volume retention as judged by reduced plasma Na^+, and decreased plasma and ventricular ANP concentrations in high calcium-fed DOC-NaCl rats. However, calcium supplementation did not affect the DOC-NaCl-induced rise in platelet $[Ca^{2+}]_i$.

3 Smooth muscle contractions of isolated mesenteric arterial rings in response to depolarization by K^+ (20–30 mM) were enhanced in DOC-NaCl-treated rats, this enhancement being abolished by concurrent oral calcium loading. The Ca^{2+} entry blocker nifedipine (10 nM) inhibited the contractions induced by K^+ (30–125 mM) more effectively in DOC-NaCl rats than in controls, while the inhibition in calcium-loaded DOC-NaCl rats was significantly greater than in controls only with 30 mM K^+.

4 The contractions of mesenteric arterial rings induced by omission of K^+ from the organ baths were used to evaluate cell membrane permeability to ions. In chemically denervated rings the onset of the gradual rise in contractile force in K^+-free medium occurred earlier, and the rate of the contraction was faster in DOC-NaCl-treated rats than in controls and high calcium-fed DOC-NaCl rats. Smooth muscle relaxation induced by 0.5 mM K^+ upon K^+-free contractions was clearly slower in DOC-NaCl rats than in controls and calcium-supplemented DOC-NaCl rats.

5 The functions of arterial smooth muscle Na^+, Ca^{2+} exchange and Ca^{2+}-ATPase were evaluated by the aortic contractions elicited by low Na^+ medium, and the subsequent relaxation responses induced by Ca^{2+}-free solution (in the presence of 5 mM caffeine, 1 μM nifedipine and 10 μM phentolamine). The rate of aortic low Na^+ contractions (evaluating Ca^{2+} influx via Na^+, Ca^{2+} exchange), as well as that of subsequent relaxations was slower in DOC-NaCl-treated rats than in controls, whether the relaxation was induced in normal (144.0 mM) or low (1.2 mM) organ bath Na^+ concentration (reflecting Ca^{2+} extrusion by both Ca^{2+}-ATPase and Na^+, Ca^{2+} exchange, and by Ca^{2+}-ATPase alone, respectively). However, in calcium-supplemented DOC-NaCl rats the aortic responses did not differ from control. The difference between the relaxation rate in normal and low Na^+ concentration in each aortic ring, representing the contribution of Na^+, Ca^{2+} exchange in these relaxations, was comparable in all groups.

6 In conclusion, calcium supplementation clearly attenuated the development of hypertension, cardiac hypertrophy, and sodium retention induced by the DOC-NaCl treatment. However, the associated rise in platelet $[Ca^{2+}]_i$ was not prevented, suggesting that in this form of experimental hypertension increased dietary calcium does not lower blood pressure by reducing $[Ca^{2+}]_i$. The results from vascular responses *in vitro* suggest that in arterial smooth muscle the DOC-NaCl treatment increased contractile sensitivity to depolarization, voltage-dependent Ca^{2+} entry and cell membrane permeability to ions, and attenuated relaxation responses and vascular Na^+, K^+-ATPase function. The results further suggest reduced ability of the cell membrane to transport Ca^{2+} (possibly via Ca^{2+}-ATPase) in DOC-NaCl hypertension. The high calcium diet opposed these alterations. The present results thus provide evidence that the antihypertensive effect of a high calcium diet in mineralocorticoid-salt hypertension is mediated by its beneficial effects on systemic sodium balance and arterial smooth muscle function.

Keywords: Arterial smooth muscle; atrial natriuretic peptide; blood pressure; dietary calcium; electrolyte balance; intracellular free calcium; mineralocorticoid-salt hypertension

Introduction

Increased dietary calcium lowers blood pressure (BP) in hypertensive patients (McCarron & Morris, 1985; Lasaridis *et al.*, 1989) and experimental animals (Resnick *et al.*, 1986; Pörsti *et al.*, 1992). However, the mechanisms underlying the antihypertensive effect of high calcium intake are yet undefined. Oral calcium loading may correct altered cellular calcium metabolism in hypertension, since in spontaneously hypertensive rats (SHR) a high calcium diet has been reported to reduce intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) (Young *et al.*, 1988; Furspan *et al.*, 1989; Pörsti *et al.*, 1992), a primary determinant of arterial contractions (Stull *et al.*, 1991). The reduction of abnormally elevated $[Ca^{2+}]_i$ could lead to an improvement in different cell functions, which has indeed been described in arterial smooth muscle from high calcium-fed SHR (Pörsti, 1992; Pörsti *et*

[1] Author for correspondence.
[2] Present address: Department of Applied Physiology, University of Freiburg, Hermann-Herder-Strasse 7, D-7800 Freiburg, Germany.

al., 1992). However, in mineralocorticoid-salt hypertension the effects of increased dietary calcium on vascular smooth muscle function are largely unexplored. They would be of interest, since calcium supplementation has been reported to decrease arterial peripheral resistance in this form of experimental hypertension (DiPette *et al.*, 1989).

Oral calcium loading has been found to promote natriuresis (Ayachi, 1979). In addition, the BP reduction induced by calcium supplementation appears to be augmented in low renin type of human essential hypertension (Lasaridis *et al.*, 1989), in sodium-volume-dependent (i.e. low renin) experimental hypertension (Resnick *et al.*, 1986), and in SHR on high sodium intake (McCarron *et al.*, 1985). We have previously shown that a high calcium diet effectively counteracts the effects of mineralocorticoid treatment in SHR (Pörsti *et al.*, 1990; 1991) by enhancing natriuresis with an accompanying decrease in plasma atrial natriuretic peptide (ANP) (Pörsti *et al.*, 1991). Thus, it is evident that oral calcium loading also has effects on sodium metabolism.

The present study investigated the effects of a high calcium diet on BP, electrolyte balance, plasma and tissue ANP, $[Ca^{2+}]_i$, and arterial smooth muscle function in one-kidney deoxycorticosterone (DOC)-NaCl hypertensive Wistar rats. This mineralocorticoid-salt treatment considerably alters the systemic and cellular electrolyte balance (Friedman & Tanaka, 1987; Morton *et al.*, 1990), and can thus be expected to disturb cellular ion fluxes across cell membrane. Furthermore, it has been suggested that calcium supplementation may decrease membrane permeability to ions (Bohr *et al.*, 1988). Therefore, the study of arterial smooth muscle responses was designed to explore effects of DOC-NaCl treatment and high calcium diet on cell membrane function.

Methods

Animals and experimental design

Male Wistar rats were housed two animals to a cage in an experimental animal laboratory room at 22°C with a 12 h light-dark cycle, and provided standard chow (containing 1.1% calcium, 0.7% sodium chloride, 0.2% magnesium; Ewos, Södertälje, Sweden) and drinking fluid (tap water) *ad libitum*. At the age of 8 weeks the left kidney was removed from all rats under ether anaesthesia. Postoperative pain was relieved with buprenorphine (Temgesic, 0.3 mg kg^{-1} twice a day, s.c.) during the first 3 days, and 2 weeks were allowed for recovery. Then the 10-week-old rats were divided into four groups of equal mean systolic BPs, which were measured from the conscious rats by the tail cuff method at 28°C (Model 129 Blood Pressure Meter: IITC Inc., Woodland Hills, Ca., U.S.A.). One group was injected weekly with DOC (Percorten M, 25 mg kg^{-1} $week^{-1}$, s.c.) and 0.7% NaCl was added to the drinking water, while the chow remained unaltered (Group DOC, $n = 13$). For the second group the DOC-NaCl treatment was combined with dietary calcium supplementation by increasing the calcium content of the chow to 2.5% (Group Ca-DOC, $n = 13$), the increment being supplied as calcium carbonate. Two additional groups were injected weekly with equal volumes of saline instead of DOC, kept on normal drinking fluid, and fed with the high calcium (Group Ca-Wistar, $n = 8$) or standard chow (Group Wistar, $n = 13$).

BP was regularly measured during the 8-week study. In study weeks 1 and 8, urine was collected for 24 h in metabolic cages, where the rats had free access to food and drinking fluid. Fluid and food consumptions were determined by weighing the bottles and chow pellets. Urine volumes were measured and samples stored at − 20°C until assayed. At the end of the study the 18-week-old rats were decapitated and exsanguinated, and blood samples were collected into chilled heparinized tubes for plasma electrolyte and platelet $[Ca^{2+}]_i$ determinations, and chilled tubes on ice containing 2.7 mM EDTA for plasma ANP assays. Blood samples for electrolyte and ANP determinations were centrifuged, and plasma stored at − 20°C until analysis; $[Ca^{2+}]_i$ measurements were carried out immediately after blood collection. After exsanguination, the hearts, mesenteric arteries and aortae were immediately excised. The experimental design of the study was approved by the Animal Experimentation Committee of the University of Tampere, Finland.

Electrolyte measurements and platelet intracellular free calcium determination

Plasma and urine concentrations of Na^+, K^+, Ca^{2+} and Mg^{2+} were measured by atomic absorption spectrophotometer (AAS; Spectr AA-30, Varian, Techtron Ltd., Victoria, Australia). For the Ca^{2+} measurements, 5 mM $LaCl_3$ was used as ionization suppressant. Samples of abdominal aortae were cleaned of adherent connective tissue, dried overnight at 100°C and weighed. Then they were ashed in a graphite oven (Naber Industrieofenbau, Bremen, Germany) for 2 h at 800°C, and the inorganic salts dissolved in 20% nitric acid for the measurement of electrolytes by AAS. Aortic tissue electrolyte contents are expressed as a concentration per g tissue dry weight. Platelet $[Ca^{2+}]_i$ was measured with a fluorescent indicator quin-2 as previously described (Pörsti *et al.*, 1992).

Atrial natriuretic peptide determinations

Left and right auricles of the hearts were removed to be used for atrial ANP measurements, and the remaining atrial tissue carefully dissected from the ventricular tissue. The ventricles were cut into superior (15–20% of total weight) and inferior parts, the latter being used for ventricular ANP determinations to avoid atrial tissue contamination. The cardiac samples were blotted dry, weighed and stored at − 70°C until assayed. ANP was extracted from plasma as previously described (Ruskoaho *et al.*, 1989), and from the cardiac tissues by the HCl method (Ruskoaho & Leppäluoto, 1988). For the radioimmunoassay (RIA) the atrial and ventricular extracts were diluted 3×10^5 and 1.2×10^3 fold, respectively, with the RIA buffer. The tissue extracts and plasma samples were then incubated in duplicates of 100 µl with 100 µl of the specific rabbit ANP antiserum in the final dilution of 1:25000. ANP was determined from plasma and tissue samples by RIA as described by Ruskoaho *et al.* (1989). Tissue ANP is expressed as concentration per mg tissue wet weight.

Arterial responses in vitro

Two standard sections (3 mm in length) of the mesenteric artery, beginning 1.5 cm distally from the mesenteric artery aorta junction, and one standard section (4 mm) of the thoracic aorta just above the diaphragm were cut from each rat. The vascular endothelium was removed from all preparations by gently rubbing with a scuffed injection needle. The rings were then placed between hooks and mounted in an organ bath chamber. The mesenteric arterial rings were bathed in physiological salt solution (PSS) (adjusted to pH 7.4 with HCl) of the following composition (mM): NaCl 119.0, $NaHCO_3$ 25.0, glucose 11.1, KCl 4.7, $CaCl_2$ 1.6, KH_2PO_4 1.2 and $MgSO_4$ 1.2. The aortic rings were bathed in a modified PSS (adjusted to pH 7.4 with Tris/HCl) containing (mM): NaCl 142.8, glucose 11.1, HEPES 10.0, KCl 4.7, $CaCl_2$ 1.6, HaH_2PO_4 1.2 and $MgSO_4$ 1.2 (Ashida *et al.*, 1989). All rings were aerated with 95% O_2: 5% CO_2, and rinsed with fresh solutions every 20 min, during which time the pH in the baths remained stable. The rings were equilibrated for 1 h at 37°C with a resting tension of 1.5 and 2.0 g for mesenteric arteries and aortas, respectively. The force of contraction was measured with an isometric force-displacement transducer and registered on a polygraph (FT03 transducers & Model 7 E Polygraph; Grass Instrument Co., Quincy, Ma., U.S.A.).

The absence of endothelium in vascular preparations was confirmed by a lack of relaxation response to 1 μM acetylcholine in 1 μM noradrenaline (NA)-precontracted arterial rings.

Mesenteric arterial preparation 1: After a 30 min stabilization period the more proximal section cut from each mesenteric artery was used to determine cumulative concentration-response curves for high concentrations of K^+. When maximal contractions after 125 mM K^+ had fully developed, the rings were rinsed with normal PSS and the subsequent relaxation was observed until resting tension was restored. After another 30 min the K^+-induced contractions were again generated in the presence of the Ca^{2+} entry blocker nifedipine (10 nM). In solutions containing high concentrations of K^+ (20–125 mM), NaCl of the normal PSS was replaced with KCl on an equimolar basis.

Mesenteric arterial preparation 2: The more distal ring of each mesenteric artery was used to study the contractile responses evoked by omission of K^+ from the organ baths and subsequent relaxations induced by re-addition of K^+. The K^+-free buffer solution (pH 7.4) was prepared by substituting KCl and KH_2PO_4 of the normal PSS with NaCl and NaH_2PO_4, respectively, on an equimolar basis. After the rings had reached a steady contraction plateau in K^+-free solution, 0.5 mM K^+ was re-added and the subsequent relaxation registered. In an additional study with Wistar rats under experimental design identical to that described above, we further investigated the mechanisms of contractions induced by the K^+-free solution. In order to eliminate the influence of endogenously released NA on these contractions, endothelium-denuded mesenteric arterial rings from these animals were subjected to chemical adrenergic denervation by the method described by Aprigliano & Hermsmeyer (1976). The rings were denervated *in vitro* by exposing them to a buffer-free solution containing 1.2 mM 6-hydroxydopamine, and vigorously gassing them with nitrogen for 15 min, followed by a 2 h recovery period in normal PSS. Thereafter, the above described K^+-free contraction procedure was performed.

Aortic preparation: The aortic rings were used to examine indirectly the vascular Na^+, Ca^{2+} exchange mechanism and Ca^{2+}-ATPase activity, using basically the procedure described by Ashida & Blaustein (1987). The preparations were first exposed to 5 mM caffeine, which induced rapid, spontaneously fading contractions. After 10 min at resting tension the modified PSS (Na^+ 144.0 mM) was changed to low Na^+ (1.2 mM) medium, and the gradually developing contractions were observed for 10 min. Thereafter the medium was replaced with Ca^{2+}-free solution containing normal Na^+ (144.0 mM), and the resultant relaxation was registered. After another 10 min at resting tension in the modified PSS the aortic rings were again contracted by low Na^+ medium, upon which relaxation was elicited by Ca^{2+}-free solution with low Na^+ concentration (1.2 mM). The low Na^+ solutions (pH 7.4) were prepared by equimolar substition of NaCl in the modified PSS with N-methyl-D-glucamine. In Ca^{2+}-free solutions $CaCl_2$ was replaced with $MgCl_2$ on an equimolar basis. All aortic contractile and relaxation responses were performed in the presence of 5 mM caffeine, 1 μM nifedipine and 10 μM phentolamine to inhibit intracellular Ca^{2+} sequestration, voltage-dependent Ca^{2+} entry, and effects of endogenously liberated NA on vascular responses, respectively.

Materials

The following compounds were used: acetoxymethyl ester of quin-2 (Aldrich Chemical Co., Milwaukee, Wi., U.S.A.), deoxycorticosterone trimethylacetate (Ciba-Geigy Ltd., Basel, Switzerland), noradrenaline hydrogentartrate (Fluka Chemie AG, Buchs SC, Switzerland), caffeine (E. Merck AG, Darmstadt, Germany), nifedipine (Orion Pharmaceutical Co., Espoo, Finland), buprenorphine hydrochloride (Reckitt & Colman, Hull, U.K.), and acetylcholine chloride, 6-hydroxydopamine hydrobromide, N-methyl-D-glucamine, phentolamine hydrochloride (Sigma Chemical Co., St. Louis, Mo., U.S.A.)

Analysis of results

The arterial contractile and relaxation responses were related to tissue dry weight, which was obtained after drying the arterial preparations overnight at 100°C. The effect of nifedipine on K^+-induced contractions was calculated by comparing the contractions in the presence of nifedipine to those without it. After re-addition of K^+ upon K^+-free contractions the reduction in smooth muscle contractile force during 1 min was considered the maximal relaxation rate, which was related to tissue dry weight. K^+ relaxation was additionally related to the pre-existing contraction induced by the K^+-free medium.

Statistical analysis was carried out by one-way analysis of variance (ANOVA) supported by Bonferroni confidence intervals using BMDP Statistical Software (Los Angeles, Ca., U.S.A.). $P<0.05$ was considered statistically significant. All results are expressed as mean ± s.e.mean.

Results

Blood pressure, heart and body weights, and plasma and tissue ANP

The mean systolic BP was stable and comparable in the Wistar and Ca-Wistar groups during the 8-week study. The DOC-NaCl treatment-induced steady rise in BP was markedly attenuated in the Ca-DOC group, whose final BP level remained about 35 mmHg lower than that of the DOC group. The final systolic BPs in the experimental groups were: Wistar 147 ± 4, Ca-Wistar 147 ± 5, DOC 201 ± 6, and Ca-DOC 165 ± 3 mmHg (Figure 1). The heart weights in the Wistar and Ca-Wistar groups were similar. The cardiac hypertrophy induced by the DOC-NaCl treatment was significantly decreasd by concurrent oral calcium loading (Table 1). The body weights in the Wistar, DOC and Ca-

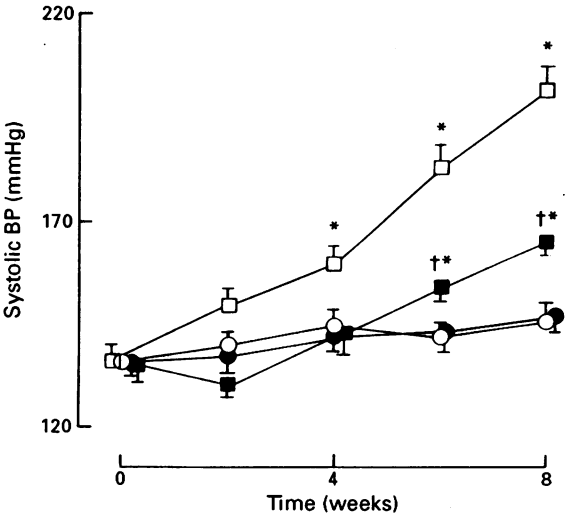

Figure 1 Systolic blood pressure (BP) in control (○, $n=13$), calcium-supplemented (●, $n=8$), deoxycorticosterone (DOC)-NaCl-treated (□, $n=13$), and calcium-supplemented DOC-NaCl-treated (■, $n=13$) Wistar rats during the 8-week study. Symbols indicate means with s.e.mean. *$P<0.05$ when compared with control rats; †$P<0.05$ when compared with DOC-NaCl-treated rats (Bonferroni test).

Table 1 Experimental group data at the end of the 8-week study

Variable	*Wistar*	*Ca-Wistar*	*DOC*	*Ca-DOC*
Body weight (g)	435 ± 9	395 ± 12*	441 ± 13	429 ± 11
Heart weight (mg)				
Right auricle	19 ± 1	19 ± 1	23 ± 2	21 ± 1
Left auricle	22 ± 2	20 ± 2	37 ± 2*	28 ± 1*†
Ventricles	868 ± 26	838 ± 37	1281 ± 41*	1049 ± 41*†
Heart:body weight ratio (mg g^{-1})	2.4 ± 0.1	2.5 ± 0.1	3.5 ± 0.1*	2.9 ± 0.1*†
Plasma electrolytes (mM)				
Na^+	121.7 ± 2.8	119.4 ± 3.3	140.6 ± 3.0*	127.9 ± 2.2†
K^+	4.19 ± 0.12	3.95 ± 0.12	4.35 ± 0.17	4.06 ± 0.12
Ca^{2+}	2.25 ± 0.04	2.54 ± 0.03*	2.44 ± 0.02*	2.38 ± 0.04*
Mg^{2+}	1.17 ± 0.03	1.09 ± 0.02*	0.96 ± 0.03*	0.89 ± 0.02*
Aortic tissue				
Na^+ (μmol g^{-1})	337 ± 22	343 ± 28	399 ± 22	347 ± 13
K^+ (μmol g^{-1})	155 ± 11	156 ± 13	154 ± 6	189 ± 12†
Na^+:K^+ ratio	2.2 ± 0.2	2.2 ± 0.2	2.6 ± 0.2	1.9 ± 0.1†
Platelet intracellular free Ca^{2+} (nM)	108 ± 4	108 ± 5	131 ± 8*	129 ± 7*

Values are mean ± s.e.mean. Ca = calcium-supplemented Wistar rats, DOC = deoxycorticosterone-NaCl-treated Wistar rats, $n = 13$ for Wistar, DOC and Ca-DOC groups, $n = 8$ for Ca-Wistar group.
*$P < 0.05$ compared with Wistar group; †$P < 0.05$ compared with DOC group (Bonferroni test).

DOC groups were comparable, but were slightly lower in the Ca-Wistar group (Table 1).

The concentrations of ANP in plasma and cardiac tissues were comparable in the Wistar and Ca-Wistar groups. The plasma ANP in the DOC group, but not in the Ca-DOC group, was significantly higher than in the Wistar group. The clear increase in ventricular ANP induced by the DOC-NaCl treatment was attenuated by calcium supplementation. The concentrations of ANP in the left atrium were decreased in both DOC and Ca-DOC groups, and in the right atrium in the Ca-DOC group (Figure 2).

Plasma and tissue electrolytes, and platelet intracellular free calcium

The DOC-NaCl treatment elevated the plasma Na^+ concentration, and the high calcium diet inhibited the elevation in the Ca-DOC group, while it did not affect plasma Na^+ in the Ca-Wistar group. Plasma K^+ levels were similar in all study groups. Both oral calcium supplementation and the DOC-NaCl treatment, and their combination, increased Ca^{2+} and decreased Mg^{2+} concentrations in plasma (Table 1). Tissue Na^+ and K^+ contents, and the Na^+:K^+ ratios in the aorta were comparable in the Wistar, Ca-Wistar and DOC groups, although tissue Na^+ tended to be increased in the DOC group. In the Ca-DOC group aortic tissue K^+ content was higher and the Na^+:K^+ ratio lower than in the DOC group (Table 1). Platelet $[Ca^{2+}]_i$, measured with quin-2, was equal in the Wistar and Ca-Wistar groups and significantly lower than in the DOC and Ca-DOC groups. Thus, the oral calcium loading did not affect the DOC-NaCl-induced rise in $[Ca^{2+}]_i$ (Table 1).

Food, fluid and electrolyte intake, and urinary electrolyte excretion

Chow consumption was comparable between the Wistar and Ca-Wistar rats, and also between the DOC and Ca-DOC rats, the consumption in the two latter being more pronounced than in the two former groups at the end of the study. Thus, the Ca-Wistar and Ca-DOC groups gained approximately 2.5 fold more Ca^{2+} than the corresponding groups on standard diet. Fluid consumption was greater in both DOC-NaCl-treated groups than in the other groups throughout the study. However, during week 8 the fluid intake (and thus Na^+ gain) in the Ca-DOC rats was attenuated to only about 60% of that in the DOC rats, Na^+ intake being increased 9.6 and 5.7 fold in the DOC and Ca-DOC groups, respectively, when compared with the Wistar group. The K^+ and Mg^{2+} gains were comparable in the

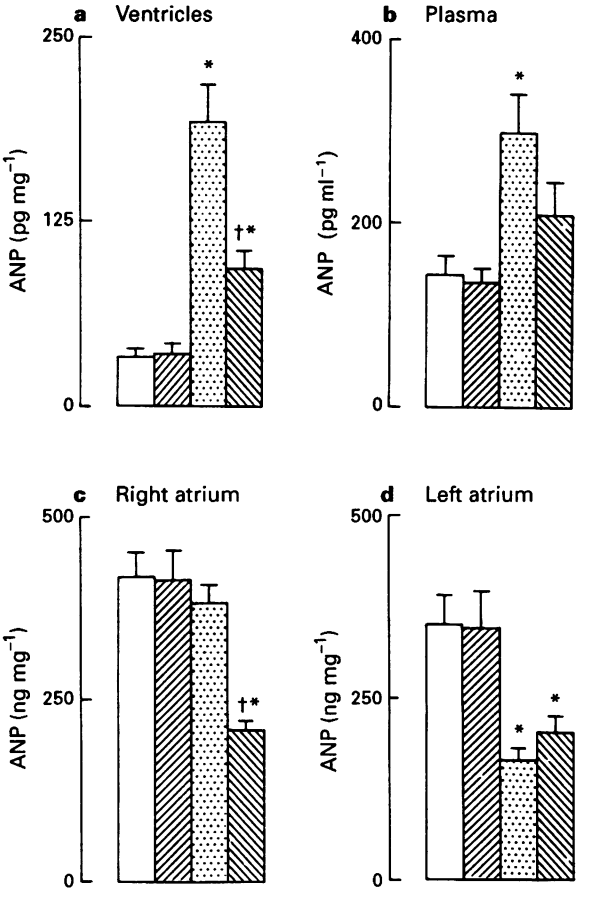

Figure 2 Atrial natriuretic peptide (ANP) concentrations in ventricular tissue (a), plasma (b), and in right (c) and left atrial tissues (d) of Wistar rats. Groups are control (□, $n = 12$), calcium-supplemented (▨, $n = 8$), deoxycorticosterone (DOC)-NaCl-treated (▩, $n = 11$), and calcium-supplemented DOC-NaCl-treated (▧, $n = 11$) Wistar rats. Columns indicate means with s.e.mean. *$P < 0.05$ when compared with control rats; †$P < 0.05$ when compared with DOC-NaCl-treated rats (Bonferroni test).

Wistar and Ca-Wistar groups, and increased in both DOC-NaCl-treated groups at the end of the study (Table 2). The urinary excretion of Na^+ was unaltered in the Ca-Wistar group, and increased in both DOC-NaCl-treated groups. Despite the lower Na^+ intake in the Ca-DOC than in the DOC group at the end of the study, Na^+ excretion did not differ significantly between these groups. K^+ excretion was proportional to the intake in all groups. Both oral calcium supplementation and DOC-NaCl treatment independently elevated urinary excretions of Ca^{2+} and Mg^{2+} to a level higher than would be expected on the basis of intakes. In the Ca-DOC group, Ca^{2+} excretion was further enhanced, whereas that of Mg^{2+} was reduced when compared to the DOC group (Table 2).

Arterial responses in vitro

The smooth muscle contractions in response to depolarization by K^+ (20–125 mM) in mesenteric arterial rings were similar in the Wistar, Ca-Wistar and Ca-DOC groups, but significantly enhanced in the DOC group with lower K^+ concentrations (20–30 mM) (Figure 3). Nifedipine (10 nM) was clearly more effective in inhibiting the K^+-evoked contractions in the DOC group than in the Wistar and Ca-Wistar groups. In the Ca-DOC group the effect of nifedipine was significantly greater than in the Wistar group only with 30 mM K^+. Thus the K^+ response in the presence of nifedipine in the Ca-DOC group was shifted towards that in the Wistar controls when compared with the DOC group (Figure 3). The washout time after maximal 125 mM K^+-induced contractions was comparable in the Wistar, Ca-Wistar and Ca-DOC groups, and shorter than in the DOC group (Table 3).

The contractions elicited by omission of K^+ from the organ baths in endothelium-denuded mesenteric arterial rings with intact adrenergic nerve-endings (representative tracing of the response shown in Figure 4) were similar in the Wistar and Ca-Wistar groups. In the DOC group the K^+-free response started earlier but the contractile rate and maximal force attained were lower than in the Wistar group. The dietary calcium supplementation during the DOC-NaCl treatment shifted the curve notably towards control (Figure 5 and Table 3). In an additional study, where the calcium supplementation again attenuated the DOC-NaCl-induced rise in BP (by 27 mmHg), the K^+-free contractions in endothelium-denuded mesenteric arterial rings were elicited after *in vitro* adrenergic denervation with 6-hydroxydopamine. When compared with the results obtained in rings with intact nerve-endings, the denervation markedly decreased the maximal K^+-free contractile force, and prolonged the time from K^+ omission to the onset of contraction and that required to reach the maximal contraction in control and calcium-supplemented Wistar rats. In DOC-NaCl-treated rats this attenuation of the K^+-free contractile rate was much smaller than in controls. In contrast to rings with intact nerve-endings, the denervated rings of DOC-NaCl-treated rats developed significantly greater maximal contractile force in shorter time, and thus a faster rate of K^+-free contraction, than those of control rats. The concurrent calcium supplementation again shifted the response towards that in controls (Table 4). The relaxations induced by 0.5 mM K^+ upon full K^+-free contractions were similar in the Wistar and Ca-Wistar groups and faster than in the DOC group, in which complete relaxation was not achieved. In the Ca-DOC group the K^+ relaxation was also slower than in the Wistar group, but nevertheless the response returned to the baseline and was clearly faster than in the DOC group (Figure 5 and Table 3).

The 5 mM caffeine-induced aortic contractions were enhanced by the DOC-NaCl treatment, but were unaffected by oral calcium loading (Table 3). The contractile force development in response to exposure to low Na^+ medium in the presence of 5 mM caffeine, 1 μM nifedipine and 10 μM phentolamine, the response thus reflecting Ca^{2+} entry via Na^+,

Table 2 Metabolic data (per 24 h) from experimental groups during study weeks 1 and 8

Variable	*Wistar*	*Ca-Wistar*	*DOC*	*Ca-DOC*
Week 1				
Chow intake (g)	26 ± 1	26 ± 1	23 ± 4	29 ± 1
Fluid intake (ml)	37 ± 1	34 ± 2	75 ± 9*	67 ± 8*
Urine volume (ml)	17 ± 1	15 ± 1	43 ± 7*	35 ± 5*
Electrolyte intake (mmol)				
Na^+	3.4 ± 0.1	3.4 ± 0.1	12.9 ± 1.6*	12.4 ± 1.1*
K^+	5.3 ± 0.2	5.3 ± 0.2	4.8 ± 0.8	5.9 ± 0.2
Ca^{2+}	7.1 ± 0.2	16.2 ± 0.6*	6.4 ± 1.1	17.8 ± 0.6*†
Mg^{2+}	2.1 ± 0.1	2.1 ± 0.1	1.9 ± 0.3	2.4 ± 0.1
Urinary excretion (mmol)				
Na^+	3.3 ± 0.2	3.1 ± 0.1	14.8 ± 1.3*	12.8 ± 1.3*
K^+	3.9 ± 0.2	4.0 ± 0.2	4.1 ± 0.4	4.2 ± 0.2
Ca^{2+}	0.09 ± 0.02	0.24 ± 0.03*	0.20 ± 0.03*	0.43 ± 0.07*†
Mg^{2+}	0.36 ± 0.06	0.73 ± 0.09*	0.90 ± 0.13*	0.86 ± 0.10*
Week 8				
Chow intake (g)	13 ± 2	15 ± 1	25 ± 2*	22 ± 2*
Fluid intake (ml)	23 ± 2	20 ± 1	100 ± 17*	53 ± 4*†
Urine volume (ml)	15 ± 2	12 ± 1	80 ± 12*	41 ± 4*†
Electrolyte intake (mmol)				
Na^+	1.7 ± 0.2	2.0 ± 0.1	16.3 ± 2.4*	9.7 ± 0.7*†
K^+	2.7 ± 0.3	3.2 ± 0.2	5.1 ± 0.4*	4.5 ± 0.3*
Ca^{2+}	3.6 ± 0.5	9.7 ± 0.5*	6.8 ± 0.5*	13.6 ± 1.0*†
Mg^{2+}	1.1 ± 0.1	1.3 ± 0.1	2.0 ± 0.2*	1.8 ± 0.1*
Urinary excretion (mmol)				
Na^+	2.5 ± 0.2	2.4 ± 0.2	17.0 ± 2.6*	12.2 ± 0.9*
K^+	3.5 ± 0.3	3.5 ± 0.2	5.4 ± 0.4*	5.0 ± 0.4*
Ca^{2+}	0.09 ± 0.01	0.31 ± 0.05*	0.40 ± 0.05*	0.69 ± 0.08*†
Mg^{2+}	0.21 ± 0.02	0.50 ± 0.03*	0.84 ± 0.13*	0.53 ± 0.03*†

Values are mean ± s.e.mean. Ca = calcium-supplemented Wistar rats, DOC = deoxycorticosterone-NaCl-treated Wistar rats, $n = 13$ for Wistar, DOC and Ca-DOC groups, $n = 8$ for Ca-Wistar group.
*$P < 0.05$ compared with Wistar group; †$P < 0.05$ compared with DOC group (Bonferroni test).

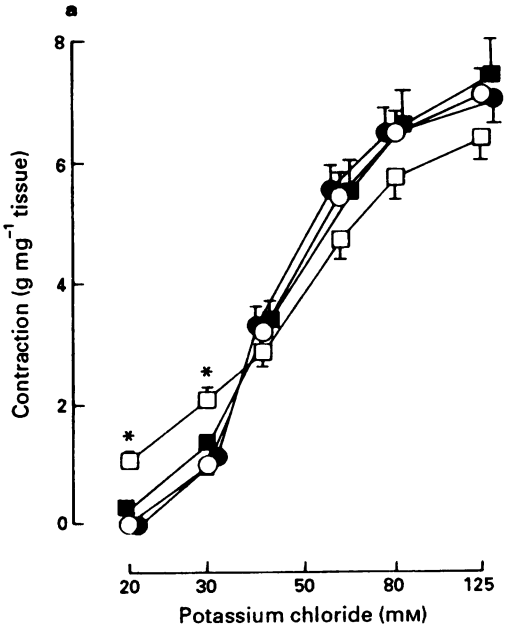

Figure 3 Concentration-response curves of Wistar rat endothelium-denuded mesenteric arterial rings to potassium chloride (a), and the effect of 10 nM nifedipine on these contractions (b). Groups are control (○, $n = 11$), calcium-supplemented (●, $n = 8$), deoxycorticosterone (DOC)-NaCl-treated (□, $n = 10$), and calcium-supplemented DOC-NaCl-treated (■, $n = 10$) Wistar rats. Symbols indicate means with s.e.mean. *$P < 0.05$ when compared with control rats (Bonferroni test).

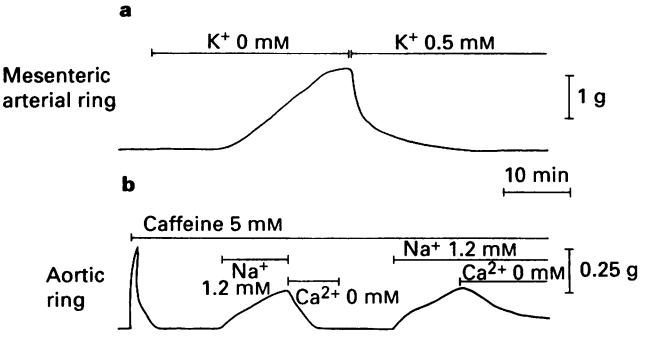

Figure 4 Representative tracings of the contractile response of an endothelium-denuded mesenteric arterial ring induced by K^+-free solution and the relaxation after the re-addition of 0.5 mM K^+ to the organ bath (a); and the contractile responses of an endothelium-denuded aortic ring induced by 5 mM caffeine, and by reduction of organ bath Na^+ concentration from 144.0 to 1.2 mM (Na^+ replaced by N-methyl-D-glucamine), and plots of subsequent aortic relaxations elicited by Ca^{2+}-free solution in normal (144.0 mM) and low (1.2 mM) organ bath Na^+ concentration. All aortic contractile and relaxation responses were performed in the presence of 5 mM caffeine, 1 μM nifedipine and 10 μM phentolamine (b).

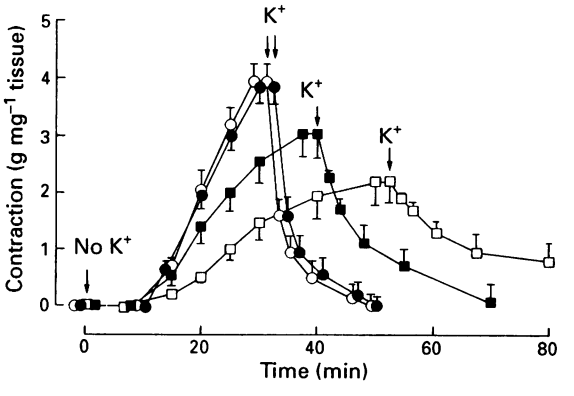

Figure 5 Contractile responses induced by K^+-free solution and subsequent relaxations in response to re-addition of 0.5 mM K^+ in Wistar rat endothelium-denuded mesenteric arterial rings with intact adrenergic nerve-endings. Groups are control (○, $n = 11$), calcium-supplemented (●, $n = 8$), deoxycorticosterone (DOC)-NaCl-treated (□, $n = 10$), and calcium-supplemented DOC-NaCl-treated (■, $n = 10$) Wistar rats. Symbols indicate means with s.e.mean. The onset of K^+-free contractions occurred earlier, and the maximal force was lower and attained later in DOC-NaCl-treated than control rats ($P < 0.05$). The concurrent calcium supplementation clearly diminished these differences. Subsequent K^+ relaxations were slower in DOC-NaCl-treated rats than in control and calcium-supplemented DOC-NaCl-treated rats ($P < 0.05$). Statistical analysis was performed by Bonferroni tests.

Ca^{2+} exchange, was similar in aortic rings from the Wistar, Ca-Wistar and Ca-DOC groups in two successive responses (representative tracing in Figure 4). In the DOC group the low Na^+ medium-induced contractions were slower than in the other groups. The rates of relaxation evoked by Ca^{2+}-free medium in a normal organ bath Na^+ concentration (144.0 mM) upon low Na^+ contractions (the relaxation evaluating Ca^{2+} extrusion by both Na^+, Ca^{2+} exchange and Ca^{2+}-ATPase) were also equal in the Wistar, Ca-Wistar and Ca-DOC groups, and attenuated in the DOC group. When relaxations were induced by Ca^{2+}-free medium in a low organ bath Na^+ concentration (1.2 mM), the responses (evaluating Ca^{2+} extrusion by Ca^{2+}-ATPase alone) were slower than those in the normal Na^+ concentration, and again slowest in the DOC group and similar in the other groups (Figure 6 and Table 3). However, the difference between the relaxation rate in normal and low organ bath Na^+ in each aortic preparation (representing the contribution of Na^+, Ca^{2+} exchange in these relaxations) was comparable in all four groups (Table 3).

Discussion

The present study shows a marked antihypertensive effect of increased dietary calcium in one-kidney DOC-NaCl hyperten-

Table 3 Parameters of arterial smooth muscle responses at the end of the 8-week study

Variable	*Wistar*	*Ca-Wistar*	*DOC*	*Ca-DOC*
Mesenteric arterial ring[a]				
Washout time after 125 mM KCl (min)	8.2 ± 0.9	7.4 ± 1.1	20.4 ± 3.0*	11.0 ± 2.3†
K^+-free contraction				
Onset of contraction (min)	9.1 ± 0.7	10.2 ± 0.7	6.8 ± 0.6*	8.0 ± 0.3
Time to maximum (min)	28.8 ± 1.5	31.5 ± 2.3	51.8 ± 3.8*	36.7 ± 3.8†
Maximal force (g mg^{-1} tissue)	4.0 ± 0.3	3.9 ± 0.3	2.3 ± 0.4*	3.1 ± 0.6
K^+ relaxation				
Time to baseline (min)	18.0 ± 2.8	16.7 ± 2.1	[b]	31.8 ± 4.5*
30 min relaxation (% of K^+-free contraction)	100 ± 0	100 ± 0	65 ± 8*	96 ± 7†
Maximal rate (g mg^{-1} tissue min^{-1})	1.18 ± 0.13	1.24 ± 0.17	0.14 ± 0.04*	0.42 ± 0.07*†
Aortic ring				
Caffeine (5 mM) contraction (mg mg^{-1} tissue)	266 ± 28	255 ± 18	410 ± 48*	417 ± 23*
Mean low Na^+ medium-induced contractile rate (mg mg^{-1} tissue min^{-1})				
1st response	11.8 ± 0.7	12.5 ± 1.0	7.4 ± 0.6*	12.0 ± 1.4†
2nd response	13.1 ± 1.6	14.0 ± 1.0	8.3 ± 0.7*	14.1 ± 1.7†
Mean relaxation rate after low Na^+ contraction (mg mg^{-1} tissue min^{-1})				
1st response (normal Na^+, Ca^{2+}-free)	26.1 ± 2.4	26.5 ± 2.4	18.0 ± 1.3*	26.0 ± 2.7†
2nd response[c] (low Na^+, Ca^{2+}-free)	8.1 ± 1.5	8.6 ± 1.0	4.1 ± 0.4*	7.2 ± 0.5†
Difference between 1st and 2nd response	17.6 ± 2.2	16.6 ± 1.5	14.2 ± 1.2	18.8 ± 2.6
2nd response: relaxation in 30 min (mg mg^{-1} tissue)	104 ± 16	104 ± 18	58 ± 6*	101 ± 7†

Values are mean ± s.e.mean. Ca = calcium-supplemented Wistar rats, DOC = deoxycorticosterone-NaCl-treated Wistar rats, $n = 9-11$ for Wistar, DOC and Ca-DOC groups, $n = 8$ for Ca-Wistar group.
*$P < 0.05$ compared with Wistar group; †$P < 0.05$ compared with DOC group (Bonferroni test).
[a]Rings with intact adrenergic nerve-endings; [b]total relaxation not achieved; [c]calculated from the first 10 min of the 2nd relaxation.

Table 4 Parameters of contractile responses induced by K^+-free solution in chemically denervated mesenteric arterial rings

Variable	*Wistar*	*Ca-Wistar*	*DOC*	*Ca-DOC*
Onset of contraction (min)	40.2 ± 9.8	44.0 ± 9.6	3.8 ± 1.2*	8.0 ± 1.7*†
Time to maximum (min)	98.1 ± 8.5	106.2 ± 4.9	72.3 ± 3.1*	81.7 ± 1.9*†
Maximal force (g mg^{-1} tissue)	1.6 ± 0.2	1.7 ± 0.2	2.2 ± 0.1*	1.9 ± 0.1

Values are mean ± s.e.mean. Ca = calcium-supplemented Wistar rats, DOC = deoxycorticosterone-NaCl-treated Wistar rats, $n = 6$ for all groups. Adrenergic denervation of the rings was performed *in vitro* with 1.2 mM 6-hydroxydopamine.
*$P < 0.05$ compared with Wistar group; †$P < 0.05$ compared with DOC group (Bonferroni test).

sive rats. Earlier investigations have also reported considerable BP reductions by supplementary calcium in sodium-volume-dependent forms of experimental hypertension (Resnick *et al.*, 1986; Yang *et al.*, 1989; Pamnani *et al.*, 1990), whereas in genetic hypertension the effect is usually only moderate (Ayachi, 1979; Pörsti, 1992; Pörsti *et al.*, 1992). This suggests that the mechanisms of action of oral calcium loading may particularly involve the correction of those abnormalities characteristic of sodium-volume-dependent hypertension (e.g. in systemic and cellular electrolyte metabolism, and membrane permeability).

In the present work, the reduction in cardiac afterload achieved by calcium supplementation was confirmed by a clear attenuation of both DOC-NaCl-induced cardiac hypertrophy and rise in ventricular ANP concentrations. The hypertension-induced ventricular hypertrophy is associated with increased synthesis, storage and release of ANP (Lattion *et al.*, 1986; Ruskoaho *et al.*, 1989; Itoh *et al.*, 1991). Thus, the attenuated rise in ventricular ANP and coincident reduction in plasma ANP levels in the Ca-DOC group indicates diminished ventricular ANP synthesis and release when compared with the DOC group. The ANP concentrations in the left atria were reduced in both DOC-NaCl-treated groups. This probably reflects increased atrial ANP release in response to increased cardiac load (Sugimoto *et al.*, 1986) because the rate of ANP synthesis in the atria of hypertrophied rat heart is known to remain fairly constant (Kinnunen *et al.*, 1991). Fujimara *et al.* (1989) reported that acute hypercalcemia is a potent stimulus for ANP release from rat hearts. Since the right atrial ANP concentrations were reduced in the Ca-DOC group but unaffected by either high calcium diet or DOC-NaCl treatment alone, the combination of local hypercalcemia in the right atrium as a result of increased calcium delivery from the intestine to venous blood, and the DOC-NaCl-induced volume load apparently augmented ANP release from the right atria of Ca-DOC rats.

The DOC-NaCl treatment caused clear sodium retention in the present study as judged by the elevated plasma N^+ concentration (and a similar tendency in arterial tissue Na^+ content) in the DOC group. Plasma and ventricular ANP concentrations were also increased in the DOC group, reflecting an attempt by the heart to reduce the volume load (Lang *et al.*, 1987). Since plasma Na^+ and ANP levels in the Ca-DOC group were comparable to those in the Wistar controls, the sodium-volume retention was attenuated by oral calcium loading. This can partially be explained by lower

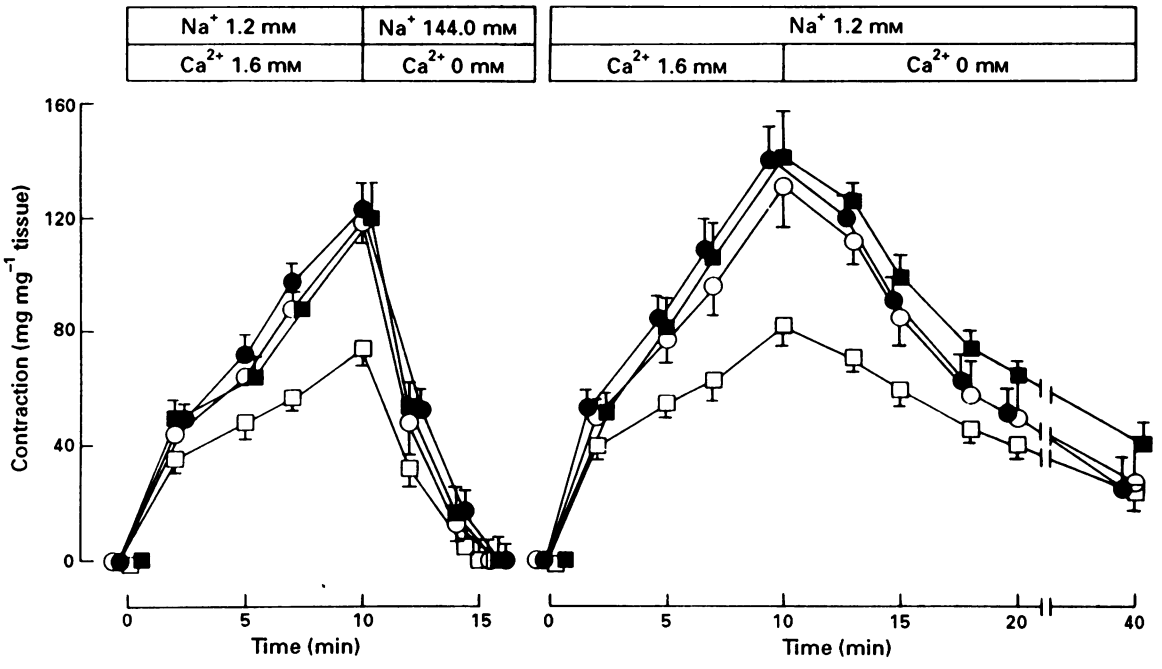

Figure 6 Contractile responses of Wistar rat endothelium-denuded aortic rings induced by reduction of organ bath Na^+ concentration from 144.0 to 1.2 mM (Na^+ replaced by N-methyl-D-glucamine), and subsequent aortic relaxations elicited by Ca^{2+}-free solution in normal (144.0 mM) and low (1.2 mM) organ bath Na^+ concentration. All responses were performed in the presence of 5 mM caffeine, 1 μM nifedipine and 10 μM phentolamine. Groups are control (○, $n = 10$), calcium-supplemented (●, $n = 8$), deoxycorticosterone (DOC)-NaCl-treated (□, $n = 10$), and calcium-supplemented DOC-NaCl-treated (■, $n = 9$) Wistar rats. Symbols indicate means with s.e.mean. The low Na^+ contractions and both subsequent Ca^{2+}-free solution-induced relaxations were slower in DOC-NaCl-treated than control rats ($P < 0.05$, Bonferroni test), while calcium-supplemented DOC-NaCl-treated rats did not differ from control rats.

sodium intake in the Ca-DOC than the DOC group. Lower sodium intake consequently decreased urinary Na^+ excretion in the Ca-DOC group, which nevertheless did not significantly differ from that in the DOC group. This suggests that additional dietary calcium enabled the Ca-DOC rats to maintain effective natriuresis at considerably lower BP levels when compared with the DOC rats. Since the pressure natriuresis was probably reduced along with the reduction of BP, the results point to the natriuretic effect of calcium supplementation previously demonstrated both in experimental animals (Ayachi, 1979; Pörsti *et al.*, 1991) and in man (Lasaridis *et al.*, 1989; Saito *et al.*, 1989). In the calcium-supplemented groups, urinary excretion of Ca^{2+} was increased more than would be expected on the basis of calcium intake, suggesting a relative enhancement of intestinal Ca^{2+} absorption during high calcium diet, a phenomenon which may be attributed to increased paracellular diffusion of Ca^{2+} in the gut (Wasserman & Fuller, 1989). Previously mineralocorticoid-salt treatment has been shown to induce hypercalciuria (Zikos *et al.*, 1986), a result also observed in the present study. Additional dietary calcium also increased the loss of Mg^{2+} into urine. This has been regarded as an adverse side-effect potentially leading to Mg^{2+} deficiency and thus impeding the beneficial effects of oral calcium loading (Evans *et al.*, 1990; Wuorela *et al.*, 1992). Accordingly, plasma Mg^{2+} levels were decreased in the calcium-supplemented groups, but no signs of compromised well-being of the animals were observed during the study, probably due to a relatively high Mg^{2+} content of the chow. Interestingly, despite its own Mg^{2+} wasting effect calcium supplementation seemed to attenuate urinary Mg^{2+} loss in the Ca-DOC group.

The concentration of cytosolic free Ca^{2+} is a primary factor determining arterial contractions (Stull *et al.*, 1991). Previously, increased dietary calcium has been found to lower both BP and $[Ca^{2+}]_i$ in SHR (Furspan *et al.*, 1989; Pörsti *et al.*, 1992; Wuorela *et al.*, 1992), giving rise to the conception that the reduction of $[Ca^{2+}]_i$ is an important antihypertensive mechanism of calcium supplementation in genetic hypertension. In the present study, platelet basal $[Ca^{2+}]_i$ was clearly higher in the DOC than the Wistar group, and was unaffected by oral calcium loading. These results are consistent with the finding that caffeine-induced aortic smooth muscle contractions, generated by Ca^{2+} release from intracellular stores (Karaki & Weiss, 1988), were also enhanced by the DOC-NaCl treatment and unaffected by the high calcium diet. Thus, the inability of calcium supplementation to decrease $[Ca^{2+}]_i$ despite a clear antihypertensive effect suggests that reduction of basal $[Ca^{2+}]_i$ is not a crucial BP-lowering mechanism of action by supplementary calcium in this type of sodium-volume-dependent hypertension.

The arterial smooth muscle contractions induced by K^+-free medium have commonly been used as a tool for studying cell membrane permeability to ions (Bohr *et al.*, 1988). The K^+-free solution inhibits vascular Na^+, K^+-ATPase and leads to Na^+ leak to the cells, which causes depolarization increasing Ca^{2+} entry through voltage-dependent channels (Mulvany, 1985). The depolarization of vascular adrenergic nerve-endings releases endogenous NA, which stimulates α-adrenoceptors and contributes to the contractile response (Vanhoutte & Lorenz, 1984). Additionally, passive Na^+ influx during Na^+, K^+-ATPase inhibition decreases the membrane Na^+ gradient and can thereby reduce Ca^{2+} extrusion by the Na^+, Ca^{2+} exchange mechanism and lead to increased intracellular Ca^{2+} (Ashida & Blaustein, 1987). However, since Na^+, Ca^{2+} exchange did not noticeably affect mesenteric arterial tone in our experimental conditions, voltage-dependent Ca^{2+} entry and endogenous release of NA are the main mechanisms of K^+-free contractions in rat mesenteric arteries (Arvola *et al.*, 1992). In the present study, the onset of K^+-free contractions occurred earlier in the DOC group than in the Wistar controls. Adrenergic denervation with

6-hydroxydopamine attenuated the K^+-free contractions less effectively in the DOC-NaCl-treated than in control rats, suggesting that these contractions are more dependent on Na^+ leak and Ca^{2+} influx to the smooth muscle cells in DOC-NaCl hypertensive than in normotensive rats, in which endogenous NA liberation is the predominant contraction mechanism. Similar finding has previously been shown in SHR (Arvola *et al.*, 1992). Furthermore, after adrenergic denervation the rate of contractile force development as well as the maximal force induced by the K^+-free solution were greater in the DOC-NaCl-treated rats than Wistar controls. The concurrent calcium supplementation partially abolished these differences. Since DOC-NaCl hypertension is known to be characterized by increased permeability to Na^+ and other substances in vascular smooth muscle (Friedman & Tanaka, 1987), the premature onset of the K^+-free contractions and the faster rate of the contractions in denervated rings in the DOC group suggest increased cell membrane permeability to ions in DOC-NaCl hypertension, which phenomenon dietary calcium supplementation appeared to reduce.

The relaxation responses following re-addition of K^+ upon K^+-free contractions indirectly evaluate vascular Na^+, K^+-ATPase activity (Hermsmeyer & Harder, 1986). In support of this, ouabain almost completely inhibits these relaxations in rat mesenteric arterial preparations (Arvola *et al.*, 1992). Na^+, K^+-ATPase has been reported to be both decreased (Songu-Mize *et al.*, 1982) and increased (Magliola *et al.*, 1986; Yang *et al.*, 1989) in DOC-NaCl hypertension. In the present study, K^+ relaxation was markedly attenuated in mesenteric arteries from the DOC group, while the response was clearly improved in the Ca-DOC group. The results support the concept of impaired vascular Na^+, K^+-ATPase function in chronic DOC-NaCl hypertension and its partial restoration during high calcium diet, a similar process being shown in calcium-supplemented SHR (Pörsti *et al.*, 1992). This indirect evidence of enhanced Na^+, K^+-ATPase by oral calcium is further supported by the reduced Na^+:K^+ ratio in aortic tissue from the Ca-DOC rats when compared with the DOC group. Since the membrane repolarization initiating the relaxation after re-addition of K^+ is probably very rapid, while complete K^+ relaxation takes several minutes, the rate of K^+ relaxation most likely also reflects general relaxation mechanisms (e.g. contractile protein dephosphorylation, Ca^{2+} sequestration and extrusion), which appear to be impaired in DOC-NaCl hypertension and enhanced by calcium supplementation. This interpretation is in agreement with the greatly prolonged washout time after maximal 125 mM KCl-induced contractions in the DOC group, but not in the Ca-DOC group.

Smooth muscle contractions induced by depolarizing the cell membrane with high concentrations of K^+ depend mainly upon Ca^{2+} influx through voltage-dependent channels, a minor proportion of the response resulting from the release of stored Ca^{2+} (Karaki & Weiss, 1988). Additionally, the release of endogenous NA during depolarization participates in these contractile responses (Fouda *et al.*, 1991). In the present study, the K^+-induced (20–125 mM) mesenteric arterial contractions were enhanced in the DOC group when using lower concentrations of K^+ (20–30 mM), and calcium supplementation abolished this enhancement. The concentration-response curves for K^+ in the presence of the dihydropyridine Ca^{2+} entry blocker, nifedipine, even more clearly differentiated the Wistar and DOC groups, nifedipine more effectively inhibiting the contractions in the DOC group. However, the inhibition by nifedipine appeared to be reduced in the Ca-DOC group. These results suggest that enhanced contractions and increased voltage-dependent Ca^{2+} entry in response to depolarization occur in DOC-NaCl hypertensive arterial smooth muscle, and that oral calcium can partially inhibit the development of these alterations. Since the effects of endogenous NA release were not eliminated in the present experiments, different adrenergic neuronal influences may have affected the results. However, the increased inhibition of these contractions by nifedipine, acting rather selectively on voltage-dependent Ca^{2+} channels, and the minor contribution of sympathetic nerve terminals on the K^+-free contractions in the DOC group suggest that increased receptor-operated Ca^{2+} entry due to enhanced NA release is not a likely explanation for the enhanced high K^+-induced contractions of the DOC rats.

The low Na^+ medium-induced vascular contractions in the presence of nifedipine, phentolamine and caffeine are considered to be due to Ca^{2+} entry via the Na^+, Ca^{2+} exchange, the dominant mechanism elevating smooth muscle $[Ca^{2+}]_i$ in these experimental conditions (Ashida & Blaustein, 1987). Nifedipine and phentolamine inhibit voltage-dependent Ca^{2+} entry activated by depolarization and receptor-operated Ca^{2+} entry stimulated by endogenous NA, respectively, and caffeine minimizes the cytosolic sequestration of influxed Ca^{2+}. The rate of low Na^+ contractions in endothelium-denuded aortic preparations was slower in the DOC than in the Ca-DOC group, which in turn did not deviate from the Wistar group. Therefore, if these contractions reflect Ca^{2+} entry into aortic smooth muscle via the Na^+, Ca^{2+} exchange, this appears to be diminished in DOC-NaCl hypertension and to be normalized by concurrent oral calcium loading. However, an alternative explanation for the attenuated low Na^+ contractions of the DOC rats may be more pronounced inhibition of aortic responses by nifedipine, since it more effectively inhibited the K^+-induced mesenteric arterial contractions in the DOC group than in the Wistar and Ca-DOC groups. Previously Ashida *et al.* (1989) have reported increased Na^+, Ca^{2+} exchange in SHR aortaes when compared with normotensive controls. Thus genetic and DOC-NaCl-induced forms of experimental hypertension appear to differ from each other in this respect. Different endothelial influences may however have affected the results, since in contrast to the present investigation the vascular endothelium was not removed in the above-mentioned study. The presence of intact endothelium may considerably depress slowly developing vascular contractions, particularly in arteries from normotensive animals (Arvola *et al.*, 1992). The role of Na^+, Ca^{2+} exchange in vascular smooth muscle function is still unclear (Matlib, 1991). This mechanism may be operative in large arteries (Ashida & Blaustein, 1987), but in smaller vessels its importance appears to be marginal (Mulvany *et al.*, 1984). Consistent with this, we have detected no contractile force development in mesenteric arterial preparations subjected to low Na^+ medium in the conditions described above (unpublished observations).

The rate of relaxation following removal of organ bath Ca^{2+} upon low Na^+ contractions reflects the ability of smooth muscle cells to pump out cytoplasmic Ca^{2+} by two potential mechanisms: Ca^{2+}-ATPase and passive Na^+, Ca^{2+} exchange (Ashida & Blaustein, 1987). The latter can mediate either Ca^{2+} entry or extrusion depending on the prevailing Na^+ and Ca^{2+} gradients across the cell membrane. At normal organ bath Na^+, the Ca^{2+}-free medium-induced relaxation response represents the combined effect of the two above mechanisms. However, at low Na^+ concentration, the relaxation is mediated by Ca^{2+}-ATPase alone, since in the absence of an inward Na^+ gradient the Na^+, Ca^{2+} exchange cannot translocate Ca^{2+} out of the cell. In the present study, the Ca^{2+}-free medium-induced relaxation responses upon low Na^+ contractions were slowest in aortic smooth muscle in the DOC group whether the organ bath Na^+ concentration was normal or low, whereas no significant differences were found between the Ca-DOC and Wistar groups. The difference between the relaxation rate in normal and low organ bath Na^+ concentration in each aortic preparation, evaluating the contribution of the Na^+, Ca^{2+} exchange to the relaxation response, was comparable in all study groups. Thus, the role of Na^+, Ca^{2+} exchange in aortic relaxation seems to be unaltered in DOC-NaCl hypertension. Instead, the slow relaxation rate induced by Ca^{2+}-free solution in aortae from DOC-NaCl-treated rats is suggestive of impaired ability of

the cell membrane to extrude Ca^{2+} via the active Ca^{2+}-ATPase, the function of which appears to be enhanced by concurrent calcium supplementation. Correspondingly, Wuorela *et al.* (1992) have previously reported that a high calcium diet activates erythrocyte Ca^{2+}-ATPase in SHR. However, since the decreased magnitude of the low Na^+ contractions in the DOC group suggests that less Ca^{2+} had entered into aortic smooth muscle cells of the DOC rats when compared with the other groups, the reduced rate of aortic relaxation in the DOC group may also be due to a decrease in the driving force for Ca^{2+} extrusion.

In conclusion, calcium supplementation clearly attenuated the development of hypertension, cardiac hypertrophy and sodium retention induced by the DOC-NaCl treatment. However, the associated rise in platelet basal $[Ca^{2+}]_i$ was not prevented, suggesting that in this form of experimental hypertension increased dietary calcium does not lower blood pressure by reducing $[Ca^{2+}]_i$. The results from vascular responses *in vitro* suggest that in arterial smooth muscle the DOC-NaCl treatment increased contractile sensitivity to depolarization, voltage-dependent Ca^{2+} entry, and cell membrane permeability to ions, and attenuated arterial relaxation and vascular Na^+, K^+-ATPase function. The results further suggest reduced ability of the cell membrane to transport Ca^{2+} (possibly via Ca^{2+}-ATPase) in DOC hypertension. The high calcium diet opposed these alterations. The present results thus provide evidence that the antihypertensive effect of a high calcium diet in mineralocorticoid-salt hypertension is mediated by its beneficial effects on systemic sodium balance and arterial smooth muscle function.

This study was supported by the Emil Aaltonen Foundation (P.A., I.P.).

References

APRIGLIANO, O. & HERMSMEYER, K. (1976). *In vitro* denervation of the portal vein and caudal artery of the rat. *J. Pharmacol. Exp. Ther.*, **94**, 325–334.

ARVOLA, P., PÖRSTI, I., VUORINEN, P., PEKKI, A. & VAPAATALO, H. (1992). Contractions induced by potassium-free solution and potassium relaxation in vascular smooth muscle of hypertensive and normotensive rats. *Br. J. Pharmacol.*, **106**, 157–165.

ASHIDA, T. & BLAUSTEIN, M.P. (1987). Regulation of cell calcium and contractility in mammalian arterial smooth muscle: the role of sodium-calcium exchange. *J. Physiol.*, **392**, 617–635.

ASHIDA, T., KURAMOCHI, M. & OMAE, T. (1989). Increased sodium-calcium exchange in arterial smooth muscle of spontaneously hypertensive rats. *Hypertension*, **13**, 890–895.

AYACHI, S. (1979). Increased dietary calcium lowers blood pressure in the spontaneously hypertensive rat. *Metabolism*, **28**, 1234–1238.

BOHR, D.F., BRUNER, C.A., LAMB, F.S. & WEBB, R.C. (1988). Physiology of vascular smooth muscle in relation to hypertension. *Acta Physiol. Scand.*, **133**, (suppl. 571), 15–24.

DiPETTE, D.J., GREILICH, P.E., KERR, N.E., GRAHAM, G.A. & HOLLAND, O.B. (1989). Systemic and regional hemodynamic effects of dietary calcium supplementation in mineralocorticoid hypertension. *Hypertension*, **13**, 77–82.

EVANS, G.H., WEAVER, C.M., HARRINGTON, D.D. & BABBS, C.F. (1990). Association of magnesium deficiency with the blood pressure-lowering effect of calcium. *J. Hypertens.*, **8**, 327–337.

FOUDA, A.-K., KAUFMANN, A., THORIN, E., HENRION, D., CAPDEVILLE-ATKINSON, C. & ATKINSON, J. (1991). The role of endogenous norepinephrine release in potassium-evoked vasoconstriction of the rat tail artery. *Eur. J. Pharmacol.*, **205**, 63–72.

FRIEDMAN, S.M. & TANAKA, M. (1987). Increased sodium permeability and transport as primary events in the hypertensive response to deoxycorticosterone acetate (DOCA) in the rat. *J. Hypertens.*, **5**, 341–345.

FUJIMURA, A., LOWRY, R.W. & KEM, D.C. (1989). Calcium infusion increases plasma atrial natriuretic factor in spontaneously hypertensive rats. *Hypertension*, **14**, 98–103.

FURSPAN, P.B., RINALDI, G.J., HOFFMAN, K. & BOHR, D.F. (1989). Dietary calcium and cell membrane abnormality in genetic hypertension. *Hypertension*, **13**, 727–730.

HERMSMEYER, K. & HARDER, D. (1986). Membrane ATPase mechanism of K^+-return relaxation in arterial muscles of stroke-prone SHR and WKY. *Am. J. Physiol.*, **250**, C557–C562.

ITOH, H., NAKAO, K., MUKOYAMA, M., ARAI, H., YAMADA, T., SAITO, Y., SHIONO, S., HOSODA, K., SHIRAKAMI, G., SUGA, S. & IMURA, H. (1991). Pathophysiological role of augmented atrial natriuretic polypeptide gene expression in DOCA-salt hypertension. *Am. J. Hypertens.*, **4**, 39–44.

KARAKI, H. & WEISS, G.B. (1988). Calcium release in smooth muscle. *Life Sci.*, **42**, 111–122.

KINNUNEN, P., TASKINEN, T., JÄRVINEN, M. & RUSKOAHO, H. (1991). Effect of phorphol ester on the release of atrial natriuretic peptide from the hypertrophied rat myocardium. *Br. J. Pharmacol.*, **102**, 453–461.

LANG, R.E., UNGER, T. & GANTEN, D. (1987). Atrial natriuretic peptide: a new factor in blood pressure control. *J. Hypertens.*, **5**, 255–271.

LASARIDIS, A.N., KAISIS, C.N., ZANANIRI, K.I., SYRGANIS, C.D. & TOURKANTONIS, A.A. (1989). Increased natriuretic ability and hypotensive effect during short-term high calcium intake in essential hypertension. *Nephron.*, **51**, 517–523.

LATTION, A.-L., MICHEL, J.-B., ARNAULD, E., CORVOL, P. & SOUBRIER, F. (1986). Myocardial recruitment during ANF mRNA increase with volume overload in the rat. *Am. J. Physiol.*, **251**, H890–H896.

MAGLIOLA, L., McMAHON, E.G. & JONES, A.W. (1986). Alterations in active Na-K transport during mineralocorticoid-salt hypertension in the rat. *Am. J. Physiol.*, **250**, C540–C546.

MATLIB, M.A. (1991). Role of sarcolemmal membrane sodium-calcium exchange in vascular smooth muscle tension. In *Sodium-Calcium Exchange*. ed. Blaustein, M.P., DiPolo, R. & Reeves, J.P. Vol. 639, pp. 531–542. New York: The New York Academy of Sciences.

McCARRON, D.A., LUCAS, P.A., SCHNEIDEMAN, R.J., LACOUR, B. & DRÜEKE, T. (1985). Blood pressure development in the spontaneously hypertensive rat after concurrent manipulations of dietary Ca^{2+} and Na^+. Relation to intestinal Ca^{2+} fluxes. *J. Clin. Invest*,. **76**, 1147–1154.

McCARRON, D.A. & MORRIS, C.D. (1985). Blood pressure response to oral calcium in persons with mild to moderate hypertension. A randomized, double-blind, placebo-controlled, crossover trial. *Ann. Intern. Med.*, **103**, (6 Pt. 1), 825–831.

MORTON, J.J., KENYON, C.J. & BEATTIE, E.C. (1990). Hormone and electrolyte changes in post-deoxycorticosterone salt hypertension in rats. *J. Hypertens.*, **8**, 1021–1026.

MULVANY, M.J. (1985). Changes in sodium pump activity and vascular contraction. *J. Hypertens.*, **3**, 429–436.

MULVANY, M.J., AALKJÆR, C. & PETERSEN, T.T. (1984). Intracellular sodium, membrane potential, and contractility of rat mesenteric small arteries. *Circ. Res.*, **54**, 740–749.

PAMNANI, M.B., CHEN, S., BRYANT, H.J., SCHOOLEY, J.F. & HADDY, F.J. (1990). Effect of increased dietary calcium on the development of reduced renal mass saline hypertension in rats. *J. Am. Coll. Nutr.*, **9**, 35–43.

PÖRSTI, I. (1992). Arterial smooth muscle contractions in spontaneously hypertensive rats on a high calcium diet. *J. Hypertens.*, **10**, 255–263.

PÖRSTI, I., ARVOLA, P., WUORELA, H., ILKKA, M., SÄYNÄVÄLAMMI, P., HUHTALA, H., METSÄ-KETELÄ, T. & VAPAATALO, H. (1990). Effects of a high calcium diet and deoxycorticosterone on vascular smooth muscle responses in spontaneously hypertensive rats. *J. Hypertens*, **8**, 835–841.

PÖRSTI, I., ARVOLA, P., WUORELA, H. & VAPAATALO, H. (1992). High calcium diet augments vascular potassium relaxation in hypertensive rats. *Hypertension*, **19**, 85–92.

PÖRSTI, I., WUORELA, H., ARVOLA, P., MÄMMI, P., NURMI, A.-K., KOISTINAHO, J., LAIPPALA, P. & VAPAATALO, H. (1991). Effects of calcium supplementation and deoxycorticosterone on plasma atrial natriuretic peptide and electrolyte excretion in spontaneously hypertensive rats. *Acta Physiol. Scand.*, **141**, 343–350.

RESNICK, L.M., SOSA, R.E., CORBETT, M.L., GERTNER, J.M., SEALEY, J.E. & LARAGH, J.H. (1986). Effect of dietary calcium on sodium volume vs. renin-dependent forms of experimental hypertension. *Trans. Assoc. Am. Physicians*, **99**, 172–179.

RUSKOAHO, H. & LEPPÄLUOTO, J. (1988). Immunoreactive atrial natriuretic peptide in ventricles, atria, hypothalmus, and plasma of genetically hypertensive rats. *Circ. Res.*, **62,** 384–394.

RUSKOAHO, H., KINNUNEN, P., TASKINEN, T., VUOLTEENAHO, O., LEPPÄLUOTO, J. & TAKALA, T.E.S. (1989). Regulation of ventricular atrial natriuretic peptide release in hypertrophied rat myocardium. Effect of exercise. *Circulation*, **80,** 390–400.

SAITO, K., SANO, H., FURUTA, Y. & FUKUZAKI, H. (1989). Effect of oral calcium on blood pressure response in salt-loaded borderline hypertensive patients. *Hypertension*, **13,** 219–226.

SONGU-MIZE, E., BEALER, S.L. & CALDWELL, R.W. (1982). Effect of AV3V lesions on development of DOCA-salt hypertension and vascular Na^+-pump activity. *Hypertension*, **4,** 575–580.

STULL, J.T., GALLAGHER, P.J., HERRING, B.P. & KAMM, K.E. (1991). Vascular smooth muscle contractile elements. Cellular regulation. *Hypertension*, **17,** 723–732.

SUGIMOTO, T., ISHII, M., HIRATA, Y., MATSUOKA, H., SUGIMOTO, T., MIYATA, A., TOSHIMORI, T., MASUDA, H., KANGAWA, K. & MATSUO, H. (1986). Increased release of atrial natriuretic polypeptides in rats with DOCA-salt hypertension. *Life Sci.*, **38,** 1351–1358.

VANHOUTTE, P.M. & LORENZ, R.R. (1984). Na^+,K^+-ATPase inhibitors and the adrenergic neuroeffector interaction in the blood vessel wall. *J. Cardiovasc. Pharmacol.*, **6,** (suppl. 1), S88–S94.

WASSERMAN, R.H. & FULLMER, C.S. (1989). On the molecular mechanism of intestinal calcium transport. *Adv. Exp. Med. Biol.*, **249,** 45–65.

WUORELA, H., ARVOLA, P., PÖRSTI, I., SILTALOPPI, E., SÄYNÄVÄLAMMI, P. & VAPAATALO, H. (1992). The effect of high calcium intake on Ca^{2+} ATPase and the tissue Na:K ratio in spontaneously hypertensive rats. *Naunyn-Schmidebergs Arch. Pharmacol.*, **345,** 117–122.

WUORELA, H., PÖRSTI, I., ARVOLA, P., MÄKYNEN, H. & VAPAATALO, H. (1992). Three levels of dietary calcium – effects on blood pressure and electrolyte balance in spontaneously hypertensive rats. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **346,** 542–549.

YANG, M.C.M., LEE, M.Y., KUO, J.S. & PANG, P.K.T. (1989). Effect of calcium diet on the vascular reactivity of DOCA-salt hypertensive rats. *Clin. Exp. Hypertens.-[A],* **11,** 501–519.

YOUNG, E.W., BUKOSKI, R.D. & MCCARRON, D.A. (1988). Calcium metabolism in experimental hypertension. *Proc. Soc. Exp. Biol. Med.*, **187,** 123–141.

ZIKOS, D., LANGMAN, C., GAFTER, U., DELAHAYE, B. & LAU, K. (1986). Chronic DOCA treatment increases Ca absorption: role of hypercalciuria and vitamine D. *Am. J. Physiol.*, **251,** E279–E284.

(Received September 4, 1992
Revised November 16, 1992
Accepted November 25, 1992)