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Effects of calcium and potassium supplements on arterial tone *in vitro* in spontaneously hypertensive rats

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1 Calcium and potassium intakes inversely correlate with blood pressure in experimental hypertension. Therefore, we examined the effects of calcium and potassium supplements alone and in combination on arterial tone in spontaneously hypertensive rats (SHR). Wistar-Kyoto (WKY) rats served as normotensive controls. Calcium and potassium contents in the control diet were both 1%, while those in supplemented chows were 3% and 3.5%, respectively. The sodium content of all diets was moderately elevated to 1.1%.

2 After 12 weeks of the study systolic blood pressures in SHR on high calcium and on high potassium diets were markedly lower (about 53 and 58 mmHg, respectively) than in hypertensive controls, while combined supplementation of these cations reduced blood pressure even further (about 69 mmHg).

3 Responses of mesenteric arterial rings *in vitro* were examined at the end of the study. Both high calcium and high potassium diets improved the impaired relaxation to acetylcholine (ACh) in SHR, while the combination of these supplements completely normalized this response. Cyclo-oxygenase inhibition by diclofenac augmented the relaxation to ACh in hypertensive controls but not in the other groups. Nevertheless, enhanced endothelium-mediated dilatation was still observed in the presence of diclofenac and the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) in all supplemented groups. Interestingly, additional blockade of Ca^{2+}-activated K^+ channels by tetraethylammonium abolished the improved relaxation to ACh in SHR on high calcium and on high potassium, but distinct responses were still observed in WKY rats and SHR on the combined supplement.

4 When hyperpolarization of smooth muscle was prevented by precontraction of the preparations with 50 mM KCl, only marginal differences were observed in the diclofenac and L-NAME-resistant relaxations to ACh between the study groups. Finally, endothelium-independent vasorelaxations of noradrenaline-precontracted rings to nitroprusside, isoprenaline and cromakalim were comparably augmented by all supplements.

5 In conclusion, the vascular mechanisms underlying the antihypertensive effect of high calcium and high potassium diets during moderately elevated sodium intake in SHR may involve enhanced arterial hyperpolarization, increased smooth muscle sensitivity to nitric oxide and decreased production of vasoconstrictor prostanoids. The administration of these cations in combination was more effective than either of them alone in reducing blood pressure and restoring arterial tone.

Keywords: Arterial smooth muscle; blood pressure; dietary calcium; dietary potassium; dietary sodium; endothelium; hyperpolarization; spontaneously hypertensive rat

Introduction

The intakes of calcium and potassium have both been shown to correlate inversely with blood pressure in clinical and experimental studies (Cutler & Brittain, 1990; Cappuccio & MacGregor 1991; Hatton & McCarron, 1994; Krishna 1994), although some contradictory results have been published as well (Sacks *et al*., 1995). In contrast, the positive association between sodium intake and blood pressure has been documented in numerous studies with human subjects and experimental animals (Aoki *et al*., 1972; Law *et al*., 1991). Interestingly, calcium as a supplement has been shown to lower blood pressure effectively in sodium volume-dependent hypertension (Arvola *et al*., 1993; Mäkynen *et al*., 1996). In addition, the antihypertensive effect of calcium has been found to be more evident during moderately elevated sodium intake in spontaneously hypertensive rats (SHR; McCarron *et al*., 1985). Parallel observations have also been made in population studies with human subjects (Hamet *et al*., 1992).

The mechanisms by which calcium and potassium supplements reduce blood pressure are not fully understood, but both vascular and nonvascular explanations have been suggested. Plausible antihypertensive mechanisms of high calcium diet include reduced plasma membrane permeability to Ca^{2+} and other cations (Hatton & McCarron, 1994), improved function of cell membrane Na^+, K^+-ATPase, reduced depolarization sensitivity and voltage-dependent Ca^{2+} entry in arterial smooth muscle (Arvola *et al*., 1993), augmented arterial sensitivity to nitric oxide (NO) and enhanced endothelium-dependent hyperpolarization of vascular smooth muscle (Mäkynen *et al*., 1996), and reduced α_1-adrenoceptor activity (Hatton *et al*., 1993). Potassium administration on the other hand, has been proposed to stimulate Na^+, K^+-ATPase in adrenergic nerve terminals and vascular smooth muscle (Haddy, 1991), increase prostacyclin synthesis (Barden *et al*.,

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1988), augment endothelium-dependent relaxation (Sugimoto *et al*., 1988) and enhance arterial compliance (Sudhir *et al*., 1993). Taken together, the above results suggest that calcium and potassium supplements decrease peripheral arterial resistance and thereby reduce blood pressure.

Since both high calcium and high potassium diets have been suggested to improve the control of vascular tone in hypertension, we investigated whether increased intake of these cations in combination would result in additional benefits in SHR ingesting a moderate sodium diet. The effects of these supplements on blood pressure, and arterial contractile and dilator responses *in vitro* were examined. Here we showed, for the first time, that endothelin-mediated dilatation could be completely normalized by combined calcium and potassium administration in this model of genetic hypertension.

Methods

Animals, experimental design and atrial natriuretic peptide (ANP) determinants

Male SHR (Okamoto-Aoki strain, $n=48$) and age-matched Wistar-Kyoto (WKY) rats ($n=12$) were obtained from Møllegaard's Breeding Centre, Ejby, Denmark. The animals were housed four to a cage in a standard experimental animal laboratory (illuminated 06 h 00 min – 18 h 00 min, temperature $+22^{\circ}$C), and had free access to drinking fluid (tap water) and food pellets. The systolic blood pressures of conscious animals held in plastic restrainers were measured at $+28^{\circ}$C by use of the tail-cuff method (Model 129 Blood Pressure Meter; IITC Inc., Woodland Hills, CA, U.S.A.) with an acclimatization period of about 30 min preceding the measurements. At 7 weeks of age, the SHR were divided into four groups ($n=12$) of equal mean systolic blood pressures. The dietary contents of Ca^{2+} and K^+ in the study groups were 1% Ca^{2+} and K^+ (control SHR), 3% Ca^{2+} and 1% K^+ (Ca-SHR), 1% Ca^{2+} and 3.5% K^+ (K-SHR) and 3% Ca^{2+} and 3.5% K^+ (CaK-SHR). All diets contained 1.1% of Na^+. WKY rats received the same diet as control SHR. The extra calcium, potassium and sodium were added to standard food pellets (Altromin no. 1314, Chr. Petersen A/S, Ringsted, Denmark) as $CaCO_3$, KCl and NaCl, respectively. Without the moderate addition of NaCl this laboratory chow can be regarded as a low Na^+ diet since it only contains 0.2% Na^+, and thus it does not correspond well with the diet consumed in most industralized societies (Law *et al*., 1991).

The supplements and indirect blood pressure measurements were continued for 12 more weeks until the animals were 19 weeks old. Thereafter, the rats were anesthetized by intraperitoneal administration of urethane (1.3 g kg^{-1}) and exsanguinated. Blood samples were drawn into chilled tubes on ice containing 2.7 mM EDTA for plasma ANP assays, thereafter the samples were centrifuged, and plasma stored at -70°C until analysis. ANP was extracted from plasma as previously described (Ruskoaho *et al*., 1989). The plasma samples were incubated in duplicates of 100 μl with 100 μl of the specific rabbit ANP antiserum in the final dilution of $1:2.5\times10^4$. ANP was determined by RIA as previously described (Ruskoaho *et al*., 1989). The hearts were removed and weighed and the superior mesenteric arteries carefully excised and cleaned of adherent connective tissue. The experimental design of the study was approved by the Animal Experimentation Committee of the University of Tampere, Finland.

Mesenteric arterial responses in vitro

Four successive standard sections (3 mm in length) of the mesenteric artery from each animal were cut, beginning 5 mm distally from the mesenteric artery-aorta junction. the endothelium of the most distal ring was removed by gently rubbing the preparation with a jagged injection needle. The rings were placed between stainless steel hooks (diameter 0.3 mm) and suspended in an organ bath chamber (volume 20 ml) in physiological salt solution (PSS) (pH 7.4) of the following composition (mM): NaCl 119.0, $NaHCO_3$ 25.0, glucose 11.1, $CaCl_2$ 1.6, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, and aerated with 95% O_2 and 5% CO_2. The rings were initially equilibrated for 30 min at 37°C with a resting tension of 1.5 g. The resting tension of 1.5 g was chosen since it was found to produce maximal contractions in all rings, and it is also widely used in this type of experiment on rat mesenteric artery (Nagao *et al*., 1992; Hwa *et al*., 1994; Mäkynen *et al*., 1995; 1996). The force of contraction was measured with an isometric force-displacement transducer and registered on a polygraph (FT03 transducer & model 7E Polygraph; Grass Instrument Co., Quincy, MA, U.S.A.). The presence of intact endothelium in vascular preparations was confirmed by clear relaxation responses to 1 μM acetylcholine (ACh) in rings that were precontracted with 1 μM noradrenaline (NA), and the absence of endothelium by the lack of this response. If any relaxation was observed in endothelium-denuded rings, the endothelium was further rubbed.

Receptor and depolarization-mediated contractions

After the equilibration period, concentration-response curves for NA and potassium chloride (KCl) were cumulatively determined. In solutions containing high concentrations of potassium (20 – 125 mM), NaCl was replaced with KCl on an equimolar basis. The next concentration of the agonist was added only when the previous level of the response was stable. After the maximal response had been reached, rings were rinsed with PSS and allowed a 20 min recovery period at resting tension. The contractions were then elicited in the presence of 3 μM diclofenac, and after this 0.1 mM N^Gnitro-L-arginine methyl ester (L-NAME) was also added to the bath and responses to NA and KCl were retested. A 30 min period was always allowed after a new drug had been introduced. We evaluated the reproducibility of the contractile responses in this arterial preparation by eliciting 4 consecutive NA cumulations under the same conditions without introduction of the drugs, and found this response highly reproducible (pD_2 of the lst *vs* the 4th response: 6.53 ± 0.12 *vs* 6.52 ± 0.06; and maximal force (g): 1.08 ± 0.26 *vs* 1.07 ± 0.26, respectively).

Endothelium-dependent relaxation after receptor-mediated precontraction

After the equilibration period, vascular responses to ACh and adenosine-5′-diphosphate (ADP) were examined. The rings were precontracted with 1 μM NA and after the contraction had fully developed increasing concentrations of the relaxing agent were cumulatively added to the organ bath. Responses to ACh were then elicited in the presence of 3 μM diclofenac, after this 0.1 mM L-NAME was also added to the bath and responses to Ach were re-tested. Finally, 1 mM tetraethylammonium (TEA) was added to the bath and the responses were again tested. We have evaluated the reproducibility of endothelium-dependent vasodilatation in this arterial preparation by eliciting 4 consecutive cumulative ACh-relaxations

under the same conditions without introduction of the drugs, and found this response highly reproducible (pD_2 of the 1st *vs* the 4th response: 7.63 ± 0.08 *vs* 7.55 ± 0.05; and maximal relaxation (%): 97.2 ± 0.50 *vs* 97.7 ± 0.63, respectively). The precontraction induced by 1 μM NA in this arterial preparation was very stable, the change in contractile force during a 20 min contraction being $1.89 \pm 1.27\%$.

Endothelium-dependent relaxation after depolarization-mediated precontraction

After the equilibration period, vascular responses to ACh and ADP were examined. The rings were precontracted with 50 mM KCl, and increasing concentrations of the relaxing agent were cumulatively added to the organ bath. Responses to ACh were then elicited in the presence of 3 μM diclofenac, after this 0.1 mM L-NAME was also added to the bath and responses to ACh were retested.

Endothelium-independent relaxations and calcium sensitivity during depolarization

After removal of endothelium and the equilibration period, cumulative relaxations to sodium nitroprusside were examined in rings precontracted with 1 μM NA and 50 mM KCl. The responses to isoprenaline and cromakalim were then cumulatively determined after precontraction with 1 μM NA. Then calcium was omitted from the buffer solution and the rings were contracted with 10 μM NA to deplete the cellular calcium stores. When the maximal response had fully developed, the rings were rinsed with Ca^{2+}-free buffer and once the resting tension had been restored the rings were challenged with 125 mM KCl. Therafter calcium was cumulatively added (0.01 – 2.5 mM) to the organ bath. The procedure was then repeated in the presence of 0.5 nM nifedipine.

Data presentation and statistical analysis of results

The maximal contractions to NA and KCl were presented in g and related to tissue dry wt. (g mg^{-1}). The EC_{50} values for NA and KCl were calculated with a computer program and presented as the negative logarithm (pD_2); these values were also used in the statistical analysis. The relaxations to ACh, nitroprusside, isoprenaline and cromakalim were presented as a percentage of the pre-existing contractile force.

Statistical analysis was carried out by one-way analysis of variance (ANOVA) supported by Bonferroni confidence intervals in the case of pairwise between-group comparisons. When the data consisted of repeated observations at successive time points ANOVA for repeated measurements was applied to investigate between-group differences. Differences were considered significant when $P < 0.05$. All results are expressed as mean $\pm$ s.e.mean. The data were analysed with BMDP statistical software.

Drugs and chemicals

The following drugs and chemicals were used: ACh cloride, sodium salt of ADP, ($\pm$)-cromakalim, ($\pm$)-isoprenaline hydrochloride, L-NAME hydrochloride, TEA chloride (Sigma Chemical Co., St. Louis, Mo., U.S.A.), diclofenac (Voltaren injection solution, Ciba-Geigy AG, Basel, Switzerland), EDTA, L-noradrenaline hydrogentartrate (Fluka Chemie AG, Buchs SG, Switzerland) and sodium nitroprusside (E. Merck AG, Darmstadt, Germany). The stock solutions of the drugs used in the *in vitro* studies were dissolved in distilled water. All solutions were freshly prepared before use and protected from light. The chemicals used in the preparation of PSS were of the highest grade available.

Results

Blood pressure, plasma ANP, and heart and body weights

The systolic blood pressures in SHR were already higher at the beginning of the study than in WKY rats. During the 12-week-long follow up, administration of either calcium, potassium or their combination, as supplements, markedly attenuated the development of hypertension in SHR. Nevertheless, blood pressures still remained higher in all SHR groups than in the WKY group. Moreover, at the end of the study (week 19) blood pressures were significantly lower in CaK-SHR than in Ca-SHR and K-SHR ($P < 0.01$, Figure 1 and Table 1).

The concentration of ANP in plasma was comparable in the SHR, Ca-SHR and WKY groups. In contrast, high potassium diet clearly elevated plasma ANP in SHR, whereas combined administration of calcium and potassium reduced the plasma concentration of ANP (Figure 2). The heart weights in WKY rats and in SHR receiving the mineral supplements were similar and lower than in control SHR (Table 1). However, all hypertensive rats given supplements gained somewhat less weight than control SHR and WKY rats, and the heart/body weight ratios did not differ between the SHR groups. In contrast, the heart/body weight ratio was clearly lower in the normotensive WKY rats when compared with all SHR groups (Table 1). No signs of a compromised well-being of the animals were observed by our experienced laboratory staff. Chow intakes were comparable in the study groups (data not shown).

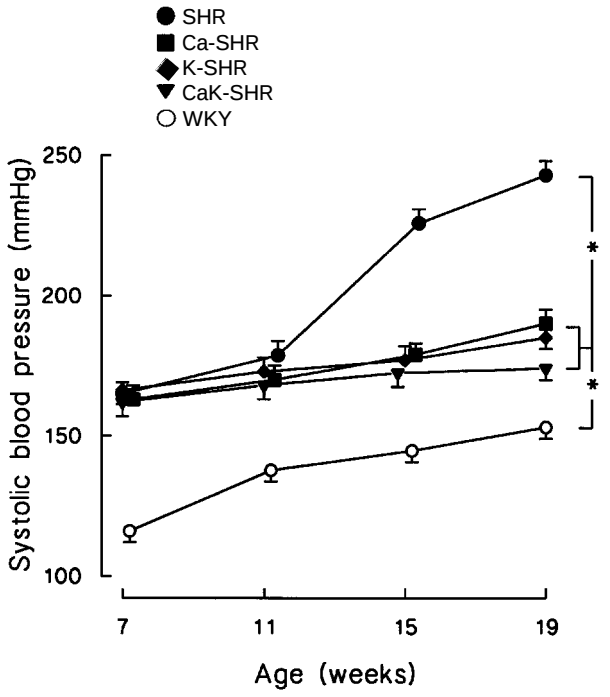

Figure 1 Blood pressures in untreated spontaneously hypertensive rats (SHR), calcium-supplemented SHR (Ca-SHR), potassium-supplemented SHR (K-SHR), calcium and potassium-supplemented SHR (CaK-SHR), and untreated Wistar-Kyoto (WKY) rats. Symbols indicate means with vertical lines showing s.e.means, $n = 10 - 12$ in each group; $^*P < 0.05$, ANOVA for repeated measurements.

Table 1 Physiological variables in the experimental groups at the close of the study

	SHR	*Ca-SHR*	*K-SHR*	*CaK-SHR*	*WKY*
Systolic blood pressure (mmHg)					
Start of treatment	165±3†	163±4†	166±4†	165±4†	116±3*
End of the study	243±4†	190±4†*	185±3†*	174±3†*	153±3*
Body wt. (g)	350±6	328±6†*	329±5†*	297±9†*	361±5
Heart wt. (g)	1.39±0.04†	1.27±0.02*	1.28±0.03*	1.22±0.03*	1.22±0.04*
Heart/body (mg g^{-1})	4.02±0.11†	3.89±0.06†	3.93±0.07†	4.12±0.16†	3.41±0.11*

Values are mean±s.e.mean, $n=10-12$ for all groups. SHR, Ca-SHR, K-SHR, CaK-SHR; untreated, calcium-supplemented, potassium-supplemented, calcium and potassium-supplemented SHR, respectively. WKY; WKY rats. *$P<0.05$ compared with SHR group, †$P<0.05$ compared with WKY group (Bonferroni test).

Mesenteric arterial responses in vitro

The endothelium-mediated relaxations of NA-precontracted mesenteric arterial rings to ACh were markedly impaired in control SHR when compared with the WKY group (Figure 3). The response to ACh was clearly improved in SHR by administration of either calcium or potassium as supplements, but the relaxation still remained less marked than in WKY rats. Interestingly, this response was completely normalized by the combined administration of these cations. Cyclo-oxygenase inhibition with diclofenac enhanced the relaxation elicited by ACh in control SHR ($P<0.001$), but did not affect the response in other groups. However, the relaxation to ACh remained diminished in control SHR when compared with the rest of the groups. The addition of the NO synthase inhibitor L-NAME to the organ bath attenuated the response to ACh in all groups ($P<0.006$ in all), and only a minute relaxation was observed in the control SHR group. TEA, an inhibitor of Ca^{2+}-activated K^+ channels (K_{Ca}; Cohen & Vanhoutte, 1995), reduced the diclofenac and L-NAME-resistant relaxation induced by ACh in all groups ($P<0.04$ in all), and almost completely abolished them in control SHR, Ca-SHR and K-SHR, while the CaK-SHR and WKY groups still showed distinct relaxations (Figure 3).

When endothelium-mediated hyperpolarization of arterial smooth muscle was eliminated by precontracting arterial rings with 50 mM KCl, the relaxations elicited by ACh in the absence and presence of diclofenac were again impaired in control SHR when compared with the other groups (Figure 4). Moreover, in the presence of diclofenac the responses to ACh were somewhat augmented in control SHR, Ca-SHR and CaK-SHR when compared with the responses elicited in the absence of diclofenac ($P<0.03$ in these groups). The addition of L-NAME markedly attenuated the relaxations to ACh in all groups ($P<0.001$ in all), and almost completely abolished them in control SHR. Interestingly, the cation-supplemented SHR showed minute diclofenac, L-NAME and depolarization-resistant relaxations to ACh (Figure 4). In addition, the results on arterial relaxation which were obtained with ADP (10 nM–0.1 mM), another endothelium-dependent agonist, were similar to those observed with ACh in both NA and KCl-precontracted vascular rings (data not shown).

The relaxation of NA or KCl-precontracted rings induced by nitroprusside, an agent that mediates arterial relaxation via the formation of exogneous NO, was markedly impaired in control SHR when compared with the WKY rats. These responses were restored in SHR by the mineral supplements. Furthermore, the supplements also clearly improved the relaxation of NA-precontracted rings to the β-adrenoceptor agonist isoprenaline and the ATP-sensitive K^+ channel (K_{ATP}) opener cromakalim (Figure 5).

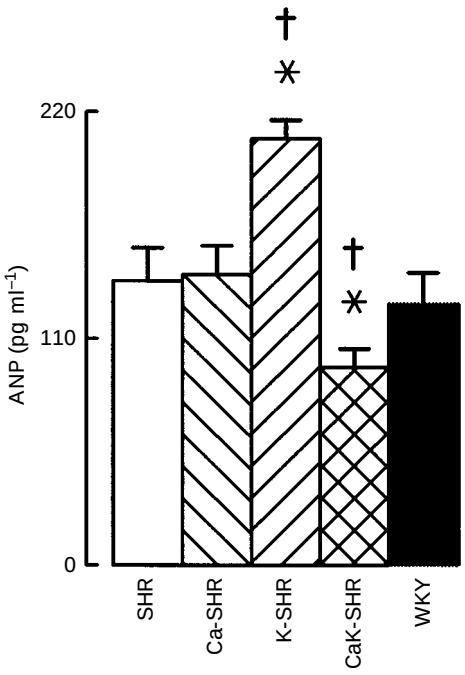

Figure 2 Plasma concentrations of ANP at the end of the study. Spontaneously hypertensive rats (SHR), calcium-supplemented SHR (Ca-SHR), potassium-supplemented SHR (K-SHR), calcium and potassium-supplemented SHR (CaK-SHR), and untreated Wistar-Kyoto (WKY) rats. Columns indicate means with s.e.means, $n=9-10$ in each group; *$P<0.05$ compared with SHR. †$P<0.05$ compared with WKY rats (Bonferroni test).

Control SHR were more sensitive to NA-induced contractions than the other groups (Table 2). The addition of diclofenac reduced sensitivity to NA in all groups ($P<0.01$ in all), and abolished the difference between control SHR and the others. However, constrictor sensitivity to KCl was comparable in the study groups, while the addition of diclofenac reduced sensitivity to KCl in all SHR groups ($P<0.001$). The addition of L-NAME did not significantly affect contractile sensitivity to NA or KCl in this study (Table 2). The maximal force generation in response to NA and KCl was comparable in the study groups, and the responses to both of these contractile agonists were correspondingly reduced by diclofenac in all groups. Moreover, the addition of L-NAME somewhat enhanced maximal force generation to NA in all SHR. In contrast, the constrictor response to KCl was significantly enhanced by L-NAME in all supplemented SHR groups but not in control SHR (Table 2). Arterial contractile sensitivity to cumulative addition of calcium during depolar-

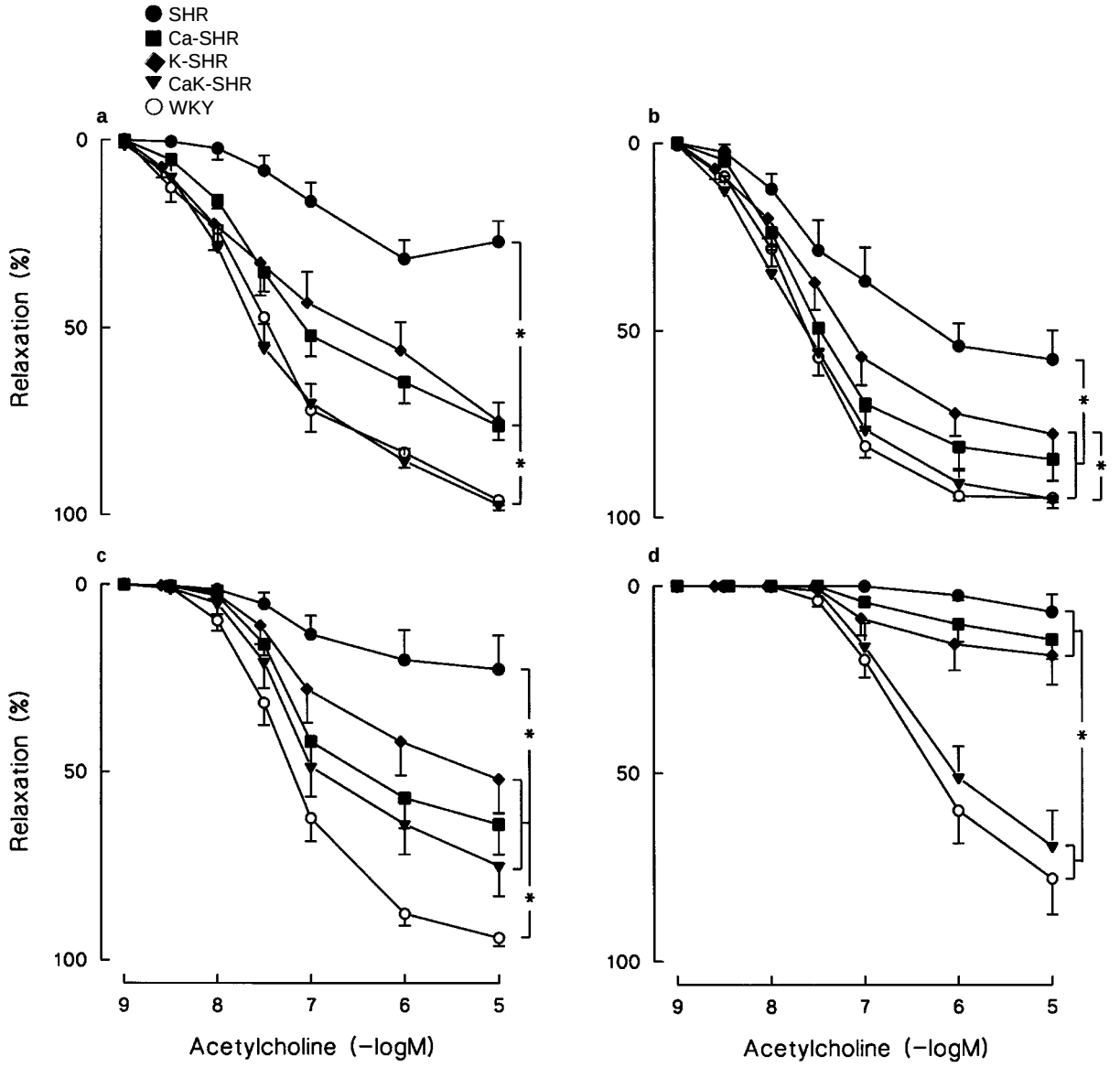

Figure 3 Relaxations to acetylcholine in noradrenaline (1 μM) precontracted isolated endothelium-intact mesenteric arterial rings from untreated spontaneously hypertensive rats (SHR) calcium-supplemented SHR (Ca-SHR), potassium-supplemented SHR (K-SHR), calcium and potassium-supplemented SHR (CaK-SHR), and untreated Wistar-Kyoto (WKY) rats. The relaxations were induced in the absence (a) and presence (b) of 3 μM diclofenac, in the presence of diclofenac and 0.1 mM N^G-nitro-L-arginine methyl ester (L-NAME; c), and in the presence of diclofenac, L-NAME and 1 mM tetraethylammonium (d). Symbols indicate means with vertical lines showing s.e.means, $n=8-10$ in each group; $^*P<0.05$, ANOVA for repeated measurements.

ization with KCl, as well as the inhibitory effect of the Ca^{2+} entry blocker nifedipine upon the response, were comparable in all study groups (data not shown).

Discussion

The present results showed that supplementation with calcium and potassium had a clear antihypertensive action in SHR which were ingesting a moderate sodium diet. Previous studies have almost uniformly shown that increased calcium intake lowers blood pressure in experimental hypertension, whereas the antihypertensive effect of high potassium diet has been less consistent (for a review see Hatton & McCarron, 1994; Krishna, 1994). Interestingly, additional antihypertensive potential could be achieved by the combination of calcium and potassium, since at the end of this study blood pressures in SHR receiving both of these cations were lower than in animals supplemented with either calcium or potassium alone.

The development of hypertension in SHR is not sodium-dependent, but moderate changes in blood pressure can be achieved by alterations in sodium intake (Aoki *et al.*, 1972). Accordingly, the present SHR on moderate sodium diet developed a more severe hypertension than in previous investigations (Mäkynen *et al.*, 1995; Sallinen *et al.*, 1996). Nevertheless, the antihypertensive effect of either calcium or potassium was not compromised by increased sodium intake, rather the effects appeared more pronounced when compared with earlier findings (Barden *et al.*, 1988; Hatton & McCarron, 1994). Previously, the lowering of blood pressure by high calcium diet has been shown to be more evident during moderately elevated sodium intake in SHR (McCarron *et al.*,

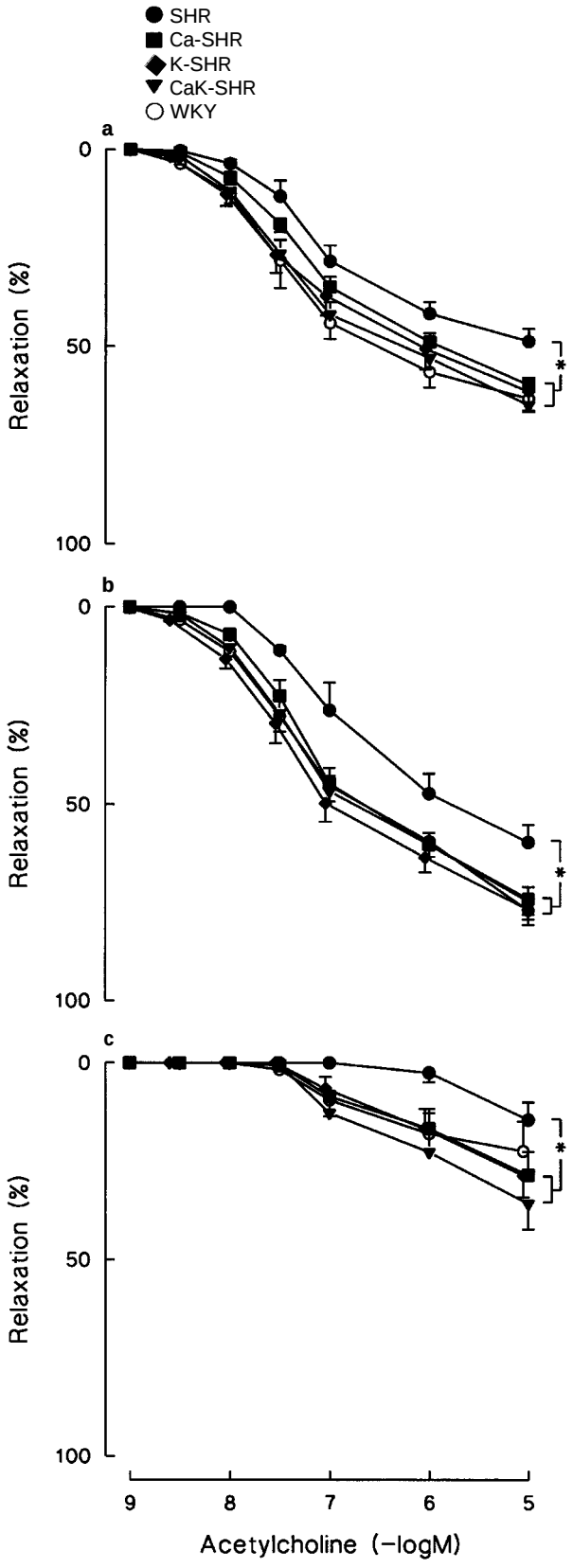

Figure 4 Relaxations to acetylcholine in KCl (50 mM) precontracted isolated endothelium-intact mesenteric arterial rings from untreated spontaneously hypertensive rats (SHR), calcium-supplemented SHR (Ca-SHR), potassium-supplemented SHR (K-SHR), calcium and potassium-supplemented SHR (CaK-SHR), and untreated Wistar-Kyoto (WKY) rats. The relaxations were induced in the absence (a) and presence (b) of 3 μM diclofenac, and in the presence of diclofenac and 0.1 mM N^G-nitro-L-arginine methyl ester (c). Symbols indicate means with vertical lines indicating s.e.means, $n=8-10$ in each group; $^*P<0.05$, ANOVA for repeated measurements.

1985), and calcium supplements have appeared especially effective under conditions of suppressed renin-angiotensin system (Arvola *et al*., 1993; Mäkynen *et al*., 1996). These findings have suggested that the impact of calcium intake on sodium metabolism is an important mediator of its cardiovascular actions (Hatton & McCarron, 1994).

The present results did not show differences in plasma ANP between SHR and WKY rats, even though hypertension-induced ventricular hypertrophy is associated with increased synthesis and release of ANP (Ruskoaho, 1992). This discrepancy can be explained by the moderately elevated sodium content of the present diet, which results in stimulated ANP release from the heart (Ruskoaho, 1992). Interestingly, high potassium diet has been shown to enhance natriuresis (Haddy, 1991), and in this study potassium administration elevated plasma ANP in SHR, which may partially explain the observed changes in blood pressure in these animals. Calcium supplements have also been suggested to increase sodium excretion (Hatton & McCarron, 1994), but recent studies have shown that the impact of high calcium diet on sodium metabolism is not mediated via ANP (Arvola *et al*., 1993; Sallinen *et al*., 1996). In this study, high calcium diet alone was without effect on ANP, whereas plasma ANP was reduced in rats receiving the combination of calcium and potassium, which may have reflected the haemodynamic changes in these animals, since plasma ANP is an index of cardiac workload (Ruskoaho, 1992). Previously, both calcium and potassium supplements have been shown to attenuate sodium retention and weight gain in essential hypertensive patients on a high sodium diet (Fujita & Ando, 1984; Saito *et al*., 1991). Correspondingly, in this study all SHR receiving the mineral diets gained less weight than control SHR. In addition, increased dietary calcium intake has even been suggested to reduce body fat content in SHR (Metz *et al*., 1988).

The heart/body weight ratios were not decreased by the present supplements. It is known that cardiac hypertrophy in SHR is not only governed by the level of blood pressure, but also by enhanced cellular responses to growth factors such as endothelin (Karam *et al*., 1996). More importantly, sodium intake is a strong and blood pressure-independent determinant of cardiac hypertrophy in experimental animals and even in human subjects (Harmsen & Leenen, 1992; Beil & Schmieder, 1995). Thus, the increased sodium content of the present diet provides an explanation why the heart/body weight ratios remained comparable in all SHR groups, despite the clear differences in blood pressure.

Previously, endothelium-dependent dilatation has been shown to be impaired in hypertension (Mäkynen *et al*., 1995; 1996). In the present investigation, administration of either calcium or potassium was accompanied by an enhancement of endothelium-mediated relaxation in SHR, this result agrees with previous findings (Sugimoto *et al*., 1988; Mäkynen *et al*., 1995). Increased potassium intake has even been shown to augment endothelium-dependent relaxation independently of its antihypertensive effect (Sugimoto *et al*., 1988). Interestingly, we found that combined administration of calcium and potassium normalized endothelium-mediated dilatation in SHR, since the relaxations to ACh and ADP were similar to the responses in the WKY rats.

Endothelial cyclo-oxygenase is a source of both vasodilator and vasoconstrictor prostanoids (Vane & Botting, 1993), and cyclo-oxygenase-dependent endothelium-derived contractile factors (EDCF) have been suggested to be involved in impaired endothelium-mediated vasomotion in SHR (Jameson *et al*., 1993). In the present study diclofenac improved the dilator response to ACh in control SHR, thus supporting the

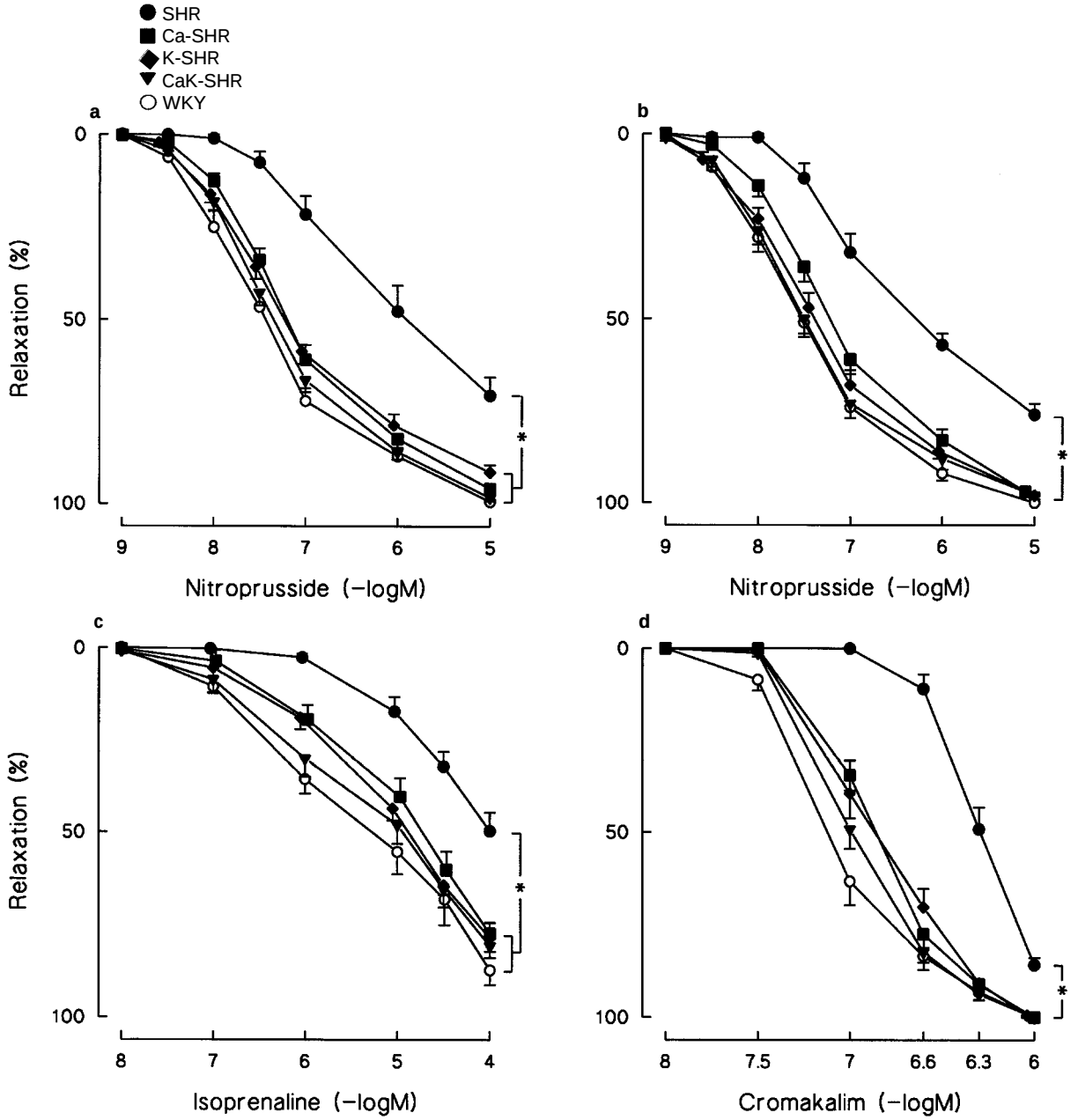

Figure 5 Relaxations to nitroprusside after precontraction with 1 μM noradrenaline (a) and 50 mM KCl (b), and relaxations to isoprenaline (c) and cromakalim (d) after precontraction with 1 μM noradrenaline. All responses were elicted in isolated endothelium-denuded mesenteric arterial rings from untreated spontaneously hypertensive rats (SHR), calcium-supplemented SHR (Ca-SHR), potassium-supplemented SHR (K-SHR), calcium and potassium-supplemented SHR (CaK-SHR), and untreated Wistar-Kyoto (WKY) rats. Symbols indicate means with vertical lines showing s.e.means, $n=8-10$ in each group; $^*P<0.05$, ANOVA for repeated measurements.

concept whereby the release of EDCF is increased in these animals. On the other hand, diclofenac was without significant effect on the response to ACh in all mineral-supplemented rats, suggesting that increased intakes of calcium and potassium diminished the production of EDCF in SHR. However, reduced EDCF release did not entirely account for the enhanced relaxations to ACh in the calcium- and potassium-supplemented rats.

Inhibition of NO synthase by L-NAME effectively diminished the relaxations to ACh in all study groups. Since the endothelium-mediated response in control SHR was nearly abolished by L-NAME, it was predominantly mediated via NO, whereas all other groups showed distinct diclofenac- and L-NAME-resistant relaxations, suggesting that endothelial products other than NO were mediating the enhanced response to ACh. Recent investigations have indicated that endothelium-mediated relaxations which remain resistant to both NO synthase and cyclo-oxygenase inhibitions are medited by another vasocative autacoid, the endothelium-derived hyperpolarizing factor (EDHF; Cohen & Vanhoutte 1995). The chemical characteristics of EDHF remain unknown, but functionally this factor is a K^+ channel opener (Cohen & Vanhoutte 1995), the action of which can be inhibited by K^+ channel blockers or by depolarizing the cell membrane with high concentrations of KCl (Adeagbo & Triggle, 1993). Interestingly, endothelium-dependent hyperpolarization has been shown to be impaired in SHR (Fujii *et al.*, 1992).

Table 2 Parameters of contractile responses of isolated endothelium-containing mesenteric arterial rings

	SHR	*Ca-SHR*	*K-SHR*	*CaK-SHR*	*WKY*
pD_2					
Noradrenaline	6.78±0.15†	6.13±0.13*	6.10±0.10*	6.23±0.09*	6.25±0.07*
with diclofenac	5.99±0.12	5.82±0.08	5.92±0.11	5.86±0.12	5.84±0.14
with L-NAME and diclofenac	5.99±0.15	5.85±0.07	5.96±0.09	6.06±0.12	5.96±0.11
Potassium chloride	1.42±0.01	1.37±0.01	1.37±0.01	1.39±0.01	1.39±0.01
with diclofenac	1.18±0.02†	1.18±0.02†	1.22±0.04	1.24±0.02	1.30±0.03*
with L-NAME and diclofenac	1.23±0.02†	1.23±0.01†	1.27±0.02	1.29±0.03	1.33±0.02*
Maximal contractile force (g mg^{-1} tissue dry wt.)					
Noradrenaline (g mg^{-1})	1.83±0.25	2.26±0.32	2.50±0.52	2.06±0.38	2.43±0.47
with diclofenac (g mg^{-1})	1.15±0.10	1.77±0.23*	1.54±0.28	1.41±0.26	1.68±0.28
change by diclofenac (%)	−27.6±9.8	−21.3±5.3	−33.1±4.3	−30.4±3.2	−31.9±12.0
with L-NAME and diclofenac (g mg^{-1})	1.18±0.17	1.88±0.23	1.75±0.31	1.69±0.32	1.95±0.32
change by L-NAME (%)	6.3±3.5†	6.9±3.9†	13.0±3.2	15.9±4.1	23.5±8.6*
Potassium chloride (g mg^{-1})	2.04±0.30	2.34±0.27	2.42±0.51	2.36±0.48	2.93±0.31
with diclofenac (g mg^{-1})	0.77±0.06†	1.18±0.17†	1.23±0.24	1.25±0.28	1.62±0.29*
change by diclofenac (%)	−48.8±10.9	−48.5±4.8	−47.9±4.1	−43.8±5.7	−43.2±7.0
with L-NAME and diclofenac (g mg^{-1})	0.81±0.08†	1.42±0.19	1.43±0.25	1.54±0.32*	1.95±0.28*
change by L-NAME (%)	1.28±6.4†	16.3±4.53*	18.03±4.6*	21.6±3.5*	16.2±5.3*

Values are mean±s.e.mean, $n=8-10$ for all groups. SHR, Ca-SHR, K-SHR, CaK-SHR; untreated, calcium-supplemented, potassium-supplemented, calcium and potassium-supplemented SHR, respectively. WKY; WKY rats. pD_2 is the negative logarithm of the concentration of agonist producing 50% of maximal contractile response. *$P<0.05$ compared with SHR group, †$P<0.05$ compared with WKY group (Bonferroni test).

The Ca^{2+}-activated K^+ channels have been found to be active during EDHF-induced relaxation (Adeagbo & Triggle, 1993), and apamin has been shown to reduce significantly the L-NAME-insensitive dilatation in the rat mesenteric artery, and apamin together with another blocker of K_{Ca}, charybdotoxin, to abolish completely these relaxations (Waldron & Garland, 1994). In the present study, inhibition of K_{Ca} by TEA reduced the diclofenac and L-NAME-resistant relaxation in all groups, and abolished the difference between control SHR and the groups supplemented with either calcium or potassium alone. This suggests that endothelium-mediated hyperpolarization via K_{Ca} was enhanced after high calcium and high potassium diets in SHR. However, SHR receiving the combined calcium and potassium supplements as well as WKY rats still showed distinct relaxations after the addition of TEA. The potential effects of TEA on endothelial K^+ channels and release of vasoactive factors may also have affected the results. Nevertheless, when hyperpolarization of arterial smooth muscle was eliminated by precontractions with KCl (as described by Adeagbo & Triggle, 1993), we found only small differences in ACh-induced relaxations between control SHR and the other groups, and the responses were similar in all mineral-supplemented SHR and the WKY rats. Collectively these findings support the view that combined administration of calcium and potassium normalized the endothelium-mediated relaxations in SHR via enhanced hyperpolarization mechanisms.

Arterial relaxations to nitroprusside were augmented in all mineral-supplemented SHR, indicating enhanced sensitivity to NO following increased calcium and potassium intakes. Recently, NO has also been shown to activate K^+ channels in vascular smooth muscle (Bolotina *et al*., 1994). However, the fact that the relaxations to nitroprusside were similarly improved whether the precontractions were induced by NA or KCl suggests that NO-mediated hyperpolarization was not playing a significant role in this response. In previous studies high calcium diet has been found to enhance vasodilatation to exogenous NO in deoxycorticosterone-NaCl hypertension (Mäkynen *et al*., 1996), whereas the relaxation to nitroprusside remained unaffected after potassium administration in stroke-prone SHR (Sugimoto *et al*., 1988).

Vasodilatation to isoprenaline is predominantly endothelium-independent via the stimulation β-adrenoceptors and the subsequent increase in cyclic AMP in smooth muscle (Bülbring & Tomita, 1987). However, isoprenaline also hyperpolarizes blood vessels via K_{ATP} and K_{Ca} in smooth muscle (Randall & McCulloch, 1995; Song & Simard, 1995). Thus, enhanced hyperpolarization could partially explain the improved relaxation to isoprenaline in the mineral-supplemented SHR in this study. Indeed, the present results whereby the responses to cromakalim, an opener of K_{ATP}, were improved in all supplemented groups further support the hypothesis that arterial hyperpolarization is augmented following increased calcium and potassium intakes. Taken together, the present results showed that both calcium and potassium supplements normalized the impaired endothelium-independent relaxation in SHR on a moderate sodium diet. The fact that the above agonists mediate vasodilatation via three different mechanisms suggests that the improvement of general vascular relaxation properties (e.g. regulation of intracellular calcium) may also have played a role in the enhanced endothelium-dependent relaxation in the mineral-supplemented groups. However, since arterial smooth muscle sensitivity to exogenous NO, β-adrenoceptor stimulation and K_{ATP} agonism was similar in all of the mineral-supplemented groups, the complete normalization of the response to ACh after the combined calcium and potassium administration suggests that the response was mediated by enhanced endothelium-dependent hyperpolarization.

Arterial contractile sensitivity to NA was lower in all mineral-supplemented SHR when compared with control SHR. This difference in contractile sensitivity between control and mineral-supplemented SHR was abolished by cyclo-oxygenase inhibition, suggesting that diminished release of vasoconstrictor prostanoids was responsible for the lower sensitivity to NA after the administration of calcium and potassium. There were no differences in arterial calcium sensitivity of KCl-induced contractions between the study

groups, or in the influence of nifedipine on these contractions. Therefore, the variations in arterial relaxation in this study cannot be attributed to differences in dihydropyridine-sensitive Ca^{2+} entry.

In conclusion, the present results showed that dietary calcium and potassium supplements effectively lowered blood pressure in SHR on moderate sodium diet. This effect was accompanied by enhanced endothelium-dependent and -independent arterial relaxation and attenuated receptor-mediated contractile sensitivity. The vascular mechanisms underlying the antihypertensive effects of these supplements may involve improved arterial hyperpolarization, increased sensitivity of smooth muscle to NO and decreased production of vasoconstrictor prostanoids. Furthermore, combined administration of calcium and potassium appeared to be more effective than either of them alone in reducing blood pressure and restoring the impaired endothelium-mediated hyperpolarization in this type of genetic hypertension.

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References

ADEAGBO, A.S.O. & TRIGGLE, C.R. (1993). Varying extracellular $[K^+]$: A functional approach to separating EDHF- and EDNO-related mechanisms in perfused rat mesenteric arterial bed. *J. Cardiovasc. Pharmacol.,* **21,** 423–429.

AOKI, K., YAMORI, Y., OSHIMA, A. & OKAMOTO, K. (1972). Effects of high or low sodium intake in spontaneously hypertensive rats. *Jpn. Circ. J.,* **36,** 539–545.

ARVOLA, P., RUSKOAHO, H. & PÖRSTI, I. (1993). Effects of high calcium diet on arterial smooth muscle function and electrolyte balance in mineralocorticoid-salt hypertensive rats. *Br. J. Pharmacol.,* **108,** 948–958.

BARDEN, A., BEILIN, L.J. & VANDONGEN, R. (1988). Effect of potassium supplementation on blood pressure and vasodilator mechanisms in spontaneously hypertensive rats. *Clin. Sci.,* **75,** 527–534.

BEIL, A.H. & SCHMIEDER, R.E. (1995). Salt intake as a determinant of cardiac hypertrophy. *Blood Press. Suppl.,* **2,** 30–34.

BOLOTINA, V.M., NAJIBI, S., PALACINO, J.J., PAGANO, P.J. & COHEN, R.A. (1994). Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. *Nature,* **368,** 850–853.

BÜLBRING, E. & TOMITA, T. (1987). Catecholamine action on smooth muscle. *Pharmacol. Rev.,* **39,** 49–96.

CAPPUCCIO, F.P. & MACGREGOR, G.A. (1991). Does potassium supplementation lower blood pressure? Meta-analysis of published trials. *J. Hypertens.,* **9,** 465–473.

COHEN, R.A. & VANHOUTTE, P.M. (1995). Endothelium-dependent hyperpolarization. Beyond nitric oxide and cyclic GMP. *Circulation,* **92,** 3337–3349.

CUTLER, J.A. & BRITTAIN, E. (1990). Calcium and blood pressure: An epidemiologic perspective. *Am. J. Hypertens.,* **3,** 137S–146S.

FUJII, K., TOMINAGA, M., OHMORI, S., KOBAYASHI, K., KOGA, T., TAKATA, Y. & FUJISHIMA, M. (1992). Decreased endothelium-dependent hyperpolarization to acetylcholine in smooth muscle of the mesenteric artery of spontaneously hypertensive rats. *Circ. Res.,* **70,** 660–669.

FUJITA, T. & ANDO, K. (1984). Hemodynamic and endocrine changes associated with potassium supplementation in sodium-loaded hypertensives. *Hypertension,* **6,** 184–192.

HADDY, F.J. (1991). Roles of sodium, potassium, calcium and natriuretic factors in hypertension. *Hypertension,* **18,** 179–183.

HAMET, P., DAIGNAULT-GELINAS, M., LAMBERT, J., LEDOUX, M., WHISSELL-GAMBIOTTI, L., BELLAVANCE, F. & MONTGEAU, E. (1992). Epidemiological evidence of an interaction between calcium and sodium intake impacting on blood pressure. A Montreal study. *Am. J. Hypertens.,* **5,** 378–385.

HWA, J.J., GHIBAUDI, L., WILLIAMS, P. & CHATTERJEE, M. (1994). Comparison of acetylcholine-dependent relaxation in large and small arteries of rat mesenteric vascular bed. *Am. J. Physiol.,* **266,** H952–H958.

HARMSEN, E. & LEENEN, F.H.H. (1992). Dietary sodium induced cardiac hypertrophy. *Can. J. Physiol. Pharmacol.,* **70,** 580–586.

HATTON, D.C. & MCCARRON, D.A. (1994). Dietary calcium and blood pressure in experimental models of hypertension. *Hypertension,* **23,** 513–530.

HATTON, D.C., SCROGIN, K.E., LEVINE, D., FELLER, D. & MCCARRON, D.A. (1993). Dietary calcium modulates blood pressure trough an α_1-adrenergic mechanism. *Am. J. Physiol.,* **264,** F234–F238.

JAMESON, M., DAI, F., LUSCHER, T., SKOPEC, J., DIEDERICH, A. & DIEDERICH, D. (1993). Endothelium-derived contracting factors in resistance arteries of young spontaneously hypertensive rats before development of overt hypertension. *Hypertension,* **21,** 280–288.

KARAM, H., HEUDES, D., BRUNEVAL, P., GONZALEZ, M.F., LOFFLER, B.M., CLOZEL, M. & CLOZEL, J.P. (1996). Endothelin antagonism in end-organ damage of spontaneously hypertensive rats. Comparison with angiotensin-converting enzyme inhibition and calcium antagonism. *Hypertension,* **28,** 379–385.

KRISHNA, G.G. (1994). Role of potassium in the pathogenesis of hypertension. *Am. J. Med. Sci.,* **307,** 21–25.

LAW, M.R., FROST, C.D. & WALD, N.J. (1991). By how much does dietary salt reduction lower blood pressure? I-Analysis of observational data among populations. *Br. Med. J.,* **302,** 811–815.

MÄKYNEN, H., KÄHÖNEN, M., ARVOLA, P., WU, X., WUORELA, H. & PÖRSTI, I. (1996). Endothelial function in deoxycorticosterone-NaCl hypertension: effect of calcium supplementation. *Circulation,* **93,** 1000–1008.

MÄKYNEN, H., KÄHÖNEN, M., ARVOLA, P., WUORELA, H., VAPAATALO, H. & PÖRSTI, I. (1995). Dietary calcium and magnesium supplements in spontaneously hypertensive rats and isolated arterial reactivity. *Br. J. Pharmacol.,* **115,** 1455–1462.

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

METZ, J.A., KARANJA, N., TOROK, J. & MCCARRON, D.A. (1988). Modification of total body fat in spontaneously hypertensive rats and Wistar-Kyoto rats by dietary calcium and sodium. *Am. J. Hypertens.,* **1,** 58–60.

NAGAO, T., ILLIANO, S. & VANHOUTTE, P.M. (1992). Heterogeneous distribution of endothelium-dependent relaxations resistant to N^G-nitro-L-arginine in rats. *Am. J. Physiol.,* **263,** H1090–H1090.

RANDALL, M.D. & MCCULLOCH, A.I. (1995). The involvement of ATP-sensitive potassium channels in β-adrenoceptor-mediated vasorelaxation in the rat isolated mesenteric arterial bed. *Br. J. Pharmacol.,* **115,** 607–612.

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

RUSKOAHO, H. (1992). Atrial natriuretic peptide: synthesis, release, and metabolism. *Pharmacol. Rev.,* **44,** 479–602.

SACKS, F.M., BROWN, L.E., APPEL, L., BORHANI, N.O., EVANS, D. & WHELTON, P. (1995). Combinations of potassium, calcium, and magnesium supplements in hyptertension. *Hypertension,* **26,** 950–956.

SAITO, K., SANO, H., FURUTA, Y., YAMANISHI, J., OMATSU, T., ITO, Y. & FUKUZAKI, H. (1991). Calcium supplementation in salt-dependent hypertension. *Contrib. Nephrol.,* **90,** 25–35.

SALLINEN, K., ARVOLA, P., RUSKOAHO, H., VAPAATALO, H. & PÖRSTI, I. (1996). High calcium diet reduces blood pressure in exercised and non-exercised hypertensive rats. *Am. J. Hypertens.,* **9,** 144–156.

SONG, Y. & SIMARD, J.M. (1995). β-Adrenoceptor stimulation activates large-conductance Ca^{2+}-activated K^{+} channels in smooth muscle cells from basilar artery of guinea pig. *Pflügers Arch. Eur. J. Physiol.,* **430,** 983–993.

SUDHIR, K., KURTZ, T.W., YOCK, P.G., CONNOLLY, A.J. & MORRIS, R.C. Jr. (1993). Potassium preserves endothelial function and enhances aortic compliance in Dahl rats. *Hypertension,* **22,** 315–322.

SUGIMOTO, T., TOBIAN, L. & GANGULI, M.C. (1988). High potassium diet protects against dysfunction of endothelial cells in Stroke-prone spontaneously hypertensive rats. *Hypertension,* **11,** 579–585.

VANE, J.R. & BOTTING, R.M. (1993). Formation by the endothelium of prostacyclin, nitric oxide and endothelin. *J. Lipid. Mediat.,* **6,** 395–404.

WALDRON, G.J. & GARLAND, C.J. (1994). Effect of potassium channel blockers on L-NAME insensitive relaxation in rat small mesenteric artery. *Can. J. Physiol. Pharmacol.,* **72,** 115.

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