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REGULATION OF SODIUM, CALCIUM AND VITAMIN D METABOLISM IN DAHL RATS ON A HIGH-SALT/LOW-POTASSIUM DIET: GENETIC AND NEURAL INFLUENCES

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SUMMARY

1. A dietary combination of high salt and low potassium (HSLK) exacerbates hypertension in Dahl salt-sensitive (DS) rats and renders previously normotensive Dahl salt-resistant (DR) rats hypertensive. In both strains, the severity of hypertension correlates with urinary calcium loss. However, the magnitude of excretory calcium losses is significantly greater in DS rats and is potentiated by chemical sympathectomy in both strains.

2. We hypothesized that a defect in vitamin D metabolism may underlie the observed strain-dependent differences in calcium balance.

3. Arterial blood pressure (ABP), water and mineral balance and serum concentrations of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2$ D_3) and 25-hydroxyvitamin D_3 ($25(OH)D_3$) were measured in intact and chemically sympathectomized (6-hydroxydopamine; 6-OHDA) DS and DR rats after 8 weeks on a HSLK diet.

4. Chronic ingestion of this diet resulted in marked and moderate levels of hypertension in DS and DR rats, respectively. The hypertension was abated and eliminated by 6-OHDA in the DS and DR strains, respectively. Independent of treatment, DS rats had significantly higher urinary excretion of calcium and reduced intestinal absorption of the ion compared with DR rats. The DS rats had significantly higher serum levels of $1,25(OH)_2$ D_3 and markedly lower serum levels of $25(OH)D_3$ than DR rats. Chemical sympathectomy tended to increase $1,25(OH)_2$ D_3 and to decrease $25(OH)D_3$ levels in both strains.

5. These data indicate a genetic difference in vitamin D metabolism between DS and DR rats. The abnormally elevated levels of $1,25(OH)_2$ D_3 in DS rats may be an appropriate compensatory response to excessive excretory calcium loss and reduced target organ sensitivity to the hormone and may, maladaptively, directly contribute to hypertension, by stimulating vascular smooth muscle contractility.

Key words: Dahl rats, genetic, hypertension, sympathetic, vitamin D.

INTRODUCTION

Dietary potassium supplementation is known to impart a degree of protection against the development of hypertension, particularly in salt-sensitive variants of the disease.[1] The multifactorial antihypertensive effect of potassium is attributed, in part, to attenuation of tonic sympathetic tone,[2] potentiation of endothelial vasodilatory activity[3] and to alterations in renal handling of electrolytes favouring increased natriuresis[4] and renal calcium conservation.[5]

We have reported previously that a dietary combination of high salt (8% NaCl) and low potassium (0.2% KCl) not only exacerbates hypertension in salt-sensitive Dahl (DS) rats, but it also renders the animals of the previously normotensive salt-resistant (DR) control strain hypertensive.[6] In both strains, the rise in arterial blood pressure (ABP) is, at least in part, due to sympathetic overactivity to the high-salt/low-potassium (HSLK) dietary intake,[7] inasmuch as prior chemical sympathectomy with 6-hydroxydopamine (6-OHDA) abrogates the hypertension in DR rats and attenuates the hypertension in DS rats. In both strains, the magnitude of HSLK-induced hypertension correlates positively with urinary excretion of calcium.[6] The severity of urinary calcium loss is further increased in both strains by 6-OHDA but, as seen in untreated animals, the calciuria is more accentuated in the DS than in DR rats.[7] In view of the well-established antihypertensive effect of calcium (for a review see Hatton and McCarron[8]), it could be inferred from these findings that a defect in calcium homeostasis may pose an additional risk factor in HSLK-induced hypertension in DS rats. In principle, dysregulation in one or several calcium-regulating mechanisms could underlie the excessive urinary calcium loss in HSLK-fed DS rats. First, the higher arterial pressure in DS rats could, in a manner analogous to pressure natriuresis, lead to increased renal calcium excretion[9] by virtue of the fact that proximal tubular transport of these two cations is coupled,[10] with diet-induced changes in the urinary excretion rate of one ion leading to parallel changes in the excretion of the other ion.[11,12] If this was the case, then DS rats, in comparison with their DR counterparts, would be expected to present a tendency towards greater natriuresis and calciuresis with consequent reduction in extracellular fluid volume (ECFV). In this context, the higher renal

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sympathetic tone in DS rats, via its direct tonic antinatriuretic/antidiuretic effects on the kidney,[13] could be viewed as exerting a potentially beneficial effect aimed at counteracting renal perfusion pressure-induced increases in salt and calcium excretion. Chemical sympathectomy would remove the renal salt-conserving activity of the sympathetic nervous system (SNS), leading to increased sodium and calcium excretion.

Alternatively, the relative calciuria in DS rats may occur in the absence of differences in sodium excretion and could be associated with an underlying defect in vitamin D metabolism. Conceivably, a reduction in biosynthesis of 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$), the biologically active hormone, a decrease in target organ responsiveness to this hormone or both could account for the less positive calcium balance induced by a HSLK diet in DS rats.[8,14,15] Alternatively, $1,25(OH)_2D_3$ levels may be appropriately elevated in the face of reduced plasma ionized calcium,[16] but the sensitivity of the renal and intestinal brush-border to the hormone may be attenuated,[15] leading to the observed reduction in calcium reabsorption. A potential functional association between vitamin D and sympathetic nervous activity in the determination of renal and cardiovascular phenotypes in HSLK-fed Dahl rats is also plausible, in light of evidence that vitamin D-dependent calcium-binding protein calbindin D-28K is pervasively colocalized in central and peripheral pre- and post-ganglionic sympathetic fibres[17,18] and is found to increase expression of the gene for the rate-limiting catecholamine synthesizing enzyme tyrosine hydroxylase[19] and to increase noradrenaline (NA) synthesis *in vivo*.[20]

The objective of the present study was to determine whether the accentuated calciuresis caused by prolonged feeding of a HSLK diet in intact and sympathectomized DS rats is caused by excessive natriuresis or whether an alteration in vitamin D status may underlie the changes in calcium excretion and arterial blood pressure caused by this diet in DS rats. Accordingly, we performed water and mineral balance studies and measured plasma concentrations of $1,25(OH)_2D_3$ and its biologically inactive percursor 25-hydroxyvitamin D ($25(OH)D_3$) in intact and in chemically sympathectomized Dahl S and R rats maintained on a HSLK diet for 8 weeks.

METHODS

Animals

Weanling male Dahl rats of both DS and DR strains were purchased from Harlan Sprague Dawley Inc. (Indianapolis, IN, USA). Animals were housed at ambient 24°C and 60% humidity in a room with a 12 : 12 h light–dark schedule and were cared for in accordance with the guidelines of the Canadian Council on Animal Care.

Dietary regimen

Two groups each of DS and DR rats were initially maintained during a 2 week acclimatization period on a low-salt/normal-potassium diet (0.008% NaCl, 4% KCl), after which they were switched to a HSLK diet (8% NaCl, 0.2% KCl) for a further 8 weeks. Beginning with the second week of low-salt feeding, one group each of DS ($n = 8$) and DR ($n = 8$) rats received injections (100 mg/kg, i.p.) of 6-OHDA (Sigma Chemical Co., St Louis, MO, USA) twice weekly to disrupt peripheral sympathetic nerve terminals.[21] The drug was dissolved in 0.001 mol/L HCl immediately prior to injection to prevent oxidation. The two remaining groups (DS $n = 9$; DR $n = 8$) received equivalent injections of vehicle. On the last day of the dietary regimen, an overnight urine sample was collected for determination of NA content (courtesy of Dr AD Baines, Department of Clinical Biochemistry, University of Toronto), as described previously.[7] Animals were then anaesthetized with Inactin (thiobutabarbital; 100 mg/kg, i.p.; Byk Gueden, Konstanz, Germany) and a femoral artery was cannulated for measurement of blood pressure and blood sampling. Blood pressure was measured using a small-volume displacement pressure transducer (model RP 1500; Narco Systems, Toronto, Ontario, Canada) connected to a MacLab/4e data acquisition system (Analog Digital Instruments, Castle Hill, NSW, Australia).

Balance studies and electrolyte analysis

For balance studies, animals were individually housed in metabolic cages. Measurement of water and food intake and urinary and faecal output of water and electrolytes was performed for the last 3 days of the HSLK dietary regimen, as described previously.[7]

Measurement of plasma $1,25(OH)_2D_3$ concentration

Serum levels of $1,25(OH)_2D_3$ were measured on purified samples (C-18Vac Elut cartridges; Varian, Harbor City, CA, USA) by a radioreceptor assay (TRK 810; Amersham, Oakville, Ontario, Canada), using vitamin D receptor isolated from calf thymus, according to the specifications of the manufacturer.

Measurement of plasma $25\text{-}(OH)D_3$

Serum levels of $25\text{-}(OH)D_3$ were measured by radioimmunoassay (Incstar, Stillwater, MN, USA) in acetonitrile-extracted samples, according to the specifications provided by the supplier.

Statistical analysis

All results are presented as the mean±SEM. Two-way analysis of variance (ANOVA) was used to compare separate and combined effects of strain and treatment on ABP, urinary NA electrolytes and serum $1,25\text{-}(OH)_2D_3$ and $25\text{-}(OH)D_3$ concentrations. Differences between groups were isolated by a multiple comparisons test coupled with the Bonferroni test. A probability level of 0.05 was deemed to indicate statistical significance.

RESULTS

The metabolic and balance data for Na^+, K^+, Ca^{2+} and Mg^{2+} for these animals have been partially reported previously.[7]

Systolic ABP and urinary Na concentrations for the different groups are shown in Table 1. The 8 weeks on the HSLK dietary regimen induced hypertension in both DS and DR rats; however, ABP was significantly more elevated in DS than in DR rats ($P < 0.0001$). Treatment with 6-OHDA significantly reduced ABP in both strains ($P < 0.0001$), although a significant pressure differential was

Table 1 Systolic arterial blood pressure and urinary noradrenaline concentrations in untreated and 6-hydroxydopamine-treated Dahl salt-sensitive and -resistant rats

	ABP (mmHg)	[Noradrenaline] (nmol/L)
DS	206±6 (9)	270±59 (3)
DR	150±1 (8)	276±41 (3)
DS (6-OHDA)	159±7 (8)	127±16 (4)
DR (6-OHDA)	123±5 (8)	117±30 (4)
2-Way ANOVA		
P, strain	< 0.0001	NS
P, treatment	< 0.0001	0.0009
P, interaction	NS	NS

Data are the mean±SEM with numbers of rats shown in parentheses.

DS, DR, Dahl salt-sensitive and -resistant, respectively; ABP, arterial blood pressure; 6-OHDA, 6-hydroxydopamine.

maintained between DS and DR rats. Basal urinary NA concentration did not differ significantly between DS and DR rats but was significantly reduced by 6-OHDA in both strains ($P < 0.0009$), thereby indicating physiologically effective sympathectomy with this treatment.

Figure 1 shows the cumulative urinary volume and urinary sodium excretion, represented as a fraction of intake for the last 3 days of the HSLK dietary regimen. There were no differences in the relative urinary excretion of water (Fig. 1a) or sodium (Fig. 1b) between DS and DR rats in either intact or in chemically sympathectomized rats. However, treatment with 6-OHDA significantly and similarly increased urinary water and sodium output in both DS and DR rats ($P < 0.0002$).

The relative cumulative urinary and faecal excretion of calcium is shown in Fig. 2. Urinary calcium excretion was significantly greater in both intact and sympathectomized DS rats than in the respective DR control rats ($P < 0.0001$; Fig. 2a). Chemical sympathectomy increased urinary calcium excretion in both strains ($P < 0.006$). No differences in faecal calcium excretion were found between intact and sympathectomized DS rats or between DS and DR rats. However, sympathectomized DR rats had a more positive faecal calcium balance than intact controls ($P < 0.02$), indicating a relative increase in calcium reabsorption.

Serum concentrations of $1,25(OH)_2D_3$ and $25(OH)D_3$ are shown in Fig. 3. Insufficient sample volume did not allow measurement of the two metabolites in all animals. The number of determinations for each group is indicated. Serum levels of $1,25(OH)_2D_3$ were significantly higher in DS rats compared with DR rats ($P < 0.05$; Fig. 1a). Treatment with 6-OHDA tended to increase the levels of $1,25(OH)_2D_3$ in both strains (Fig. 1a), but this did not reach

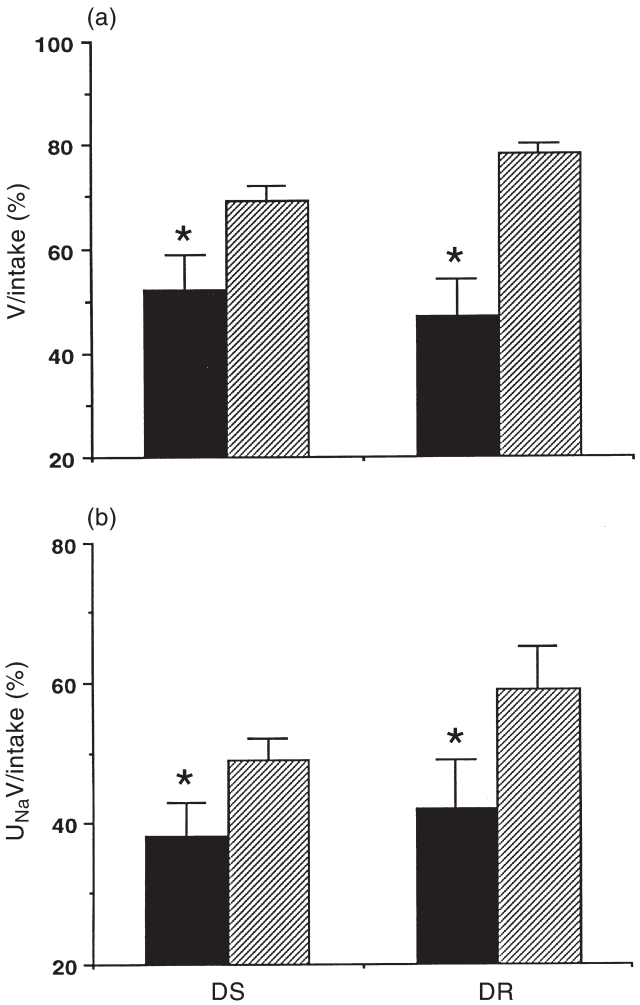

Fig. 1 Relative cumulative urinary excretion of (a) water and (b) sodium as a ratio of dietary intake for the last 3 days of an 8 week high-sodium/low-potassium dietary regimen in untreated (control; ■) and 6-hydroxydopamine-treated (6-OHDA; ▨) Dahl salt-sensitive (DS; control $n = 9$; 6-OHDA $n = 8$) and Dahl salt-resistant (DR; $n = 8$) rats. Statistically significant differences in urinary water and sodium excretion were found between untreated and 6-OHDA-treated DS rats and between untreated and 6-OHDA-treated DR rats (*$P < 0.0002$, 0.02 for water and sodium, respectively). $U_{Na}V$, urinary sodium excretion; V, urinary volume.

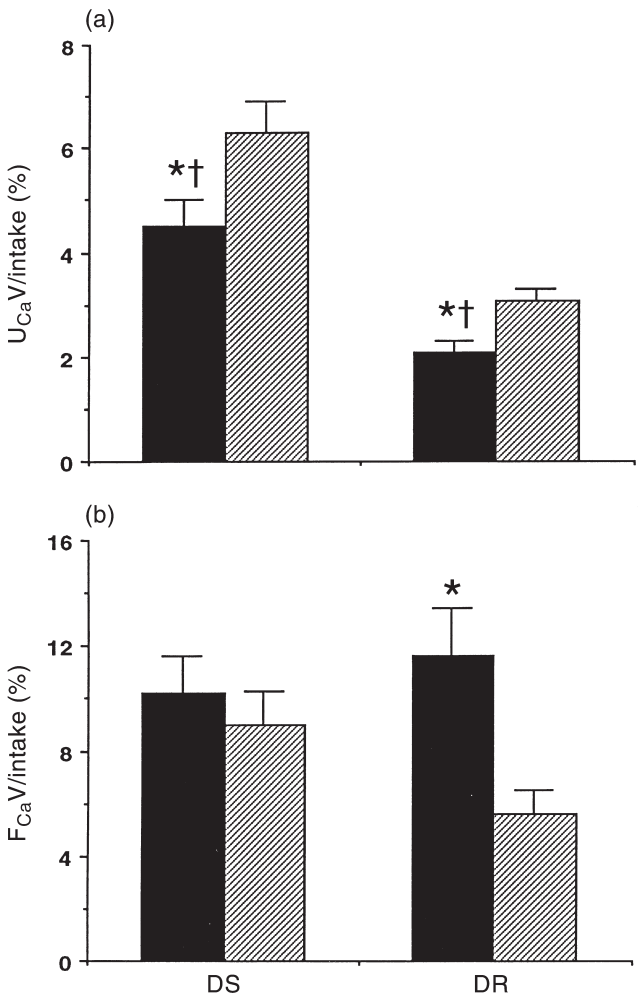

Fig. 2 Relative cumulative (a) urinary and (b) faecal excretion of calcium as a ratio of dietary intake for the last 3 days of an 8 week high-sodium/low-potassium dietary regimen in untreated (control, ■) and 6-hydroxydopamine-treated (6-OHDA; ▨) Dahl salt-sensitive (DS; control $n = 9$; 6-OHDA $n = 8$) and Dahl salt-resistant (DR; $n = 8$) rats. Significant differences in urinary calcium excretion were found between untreated and 6-OHDA-treated DS rats and between untreated and 6-OHDA-treated DR rats (*$P < 0.0001$) and between DS and DR rats in both treated and 6-OHDA-treated conditions ($^{\dagger}P = 0.006$). Statistical significant differences in faecal calcium excretion were found between untreated and 6-OHDA-treated DR rats (*$P = 0.02$). $U_{Ca}V$, urinary calcium excretion; $F_{Ca}V$, faecal calcium excretion.

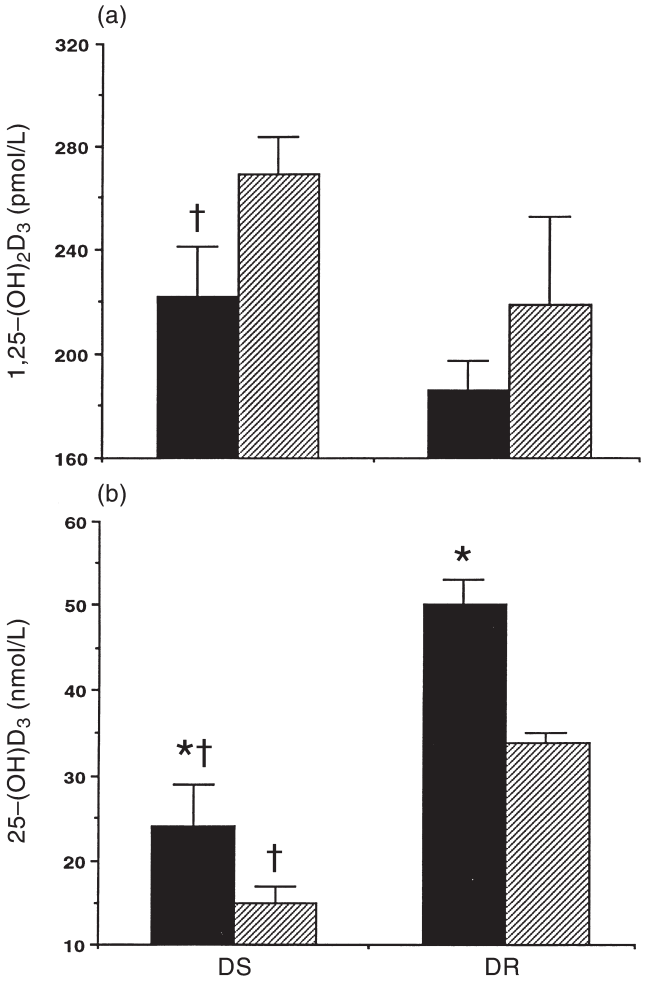

Fig. 3 Serum concentrations of (a) 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) and (b) 25-hydroxyvitamin D_3 ($25(OH)D_3$) in untreated (■) and 6-hydroxydopamine (6-OHDA)-treated (▨) Dahl salt-sensitive and -resistant (DS and DR, respectively) rats. Statistically significant differences in serum $1,25(OH)_2D_3$ concentrations were found between untreated DS and DR rats ($^{\dagger}P < 0.05$; $n = 7$). Significant differences in serum $25(OH)D_3$ were found between untreated and 6-OHDA-treated conditions in DS and DR rats ($^{*}P < 0.0001$; $n = 4$) and between DS and DR rats in both untreated and 6-OHDA-treated conditions ($^{\dagger}P < 0.0001$; $n = 4$).

statistical significance ($P < 0.07$). In contrast with $1,25(OH)_2D_3$, serum concentrations of $25(OH)D_3$ were significantly lower in DS than DR rats ($P < 0.0001$) and were significantly reduced by 6-OHDA in both strains ($P = 0.0003$; Fig. 1b).

DISCUSSION

Dahl salt-sensitive rats on a high-salt diet are known to have an inherent defect in calcium metabolism that results in excessive calcium loss.[6,22] Dietary potassium restriction in combination with a high salt intake potentiates excretory calcium loss, even in DR rats.[6] However, the degree of calciuresis is markedly elevated in DS rats[6] and is exacerbated by chronic peripheral chemical sympathectomy. Is the relative calciuria in DS rats attributable to an increase in renal sodium excretion? The present study shows similar rates of sodium excretion in DS and DR rats despite significantly elevated basal blood pressure in the former strain, thus rendering natriuresis as an unlikely mechanism for the excessive urinary calcium loss in DS rats. The increase in renal perfusion pressure in DS rats *per se* would have been expected to increase sodium excretion via pressure natriuresis[23] and, concomitantly, to increase calcium excretion.[10] Clearly, the potential effect of renal perfusion pressure on renal sodium and calcium excretion appears to be adequately counteracted by a salt-conserving mechanism(s). Blunting of the pressure natriuresis has previously been reported by Roman to occur in DS rats, even at the prehypertensive stage.[24] Our findings indicate that chemical sympathectomy significantly and similarly increases urinary water, sodium and calcium in DS and DR rats, suggesting that the HSLK-induced increase in sympathetic tone may indirectly exert a beneficial anticalciuretic effect by restraining pressure natriuresis via its direct tubular antinatriuretic and haemodynamic effects in the kidney.[13]

Alternatively, a genetic alteration in vitamin D metabolism may account for the differences in calcium handling between the HSLK-fed DS and DR rats. The current results show that plasma levels of $1,25(OH)_2D_3$ are significantly higher in both intact and chemically sympathectomized DS rats than in similarly maintained DR rats. This finding suggests that the salt-sensitive strain is capable of adjusting its rate of $1,25(OH)_2D_3$ biosynthesis to underlying changes in calcium balance. In contrast, whereas the sympathectomized DR rats show an appropriate reduction in faecal calcium excretion, the DS rats do not, despite significantly less positive calcium balance. This suggests that target organ hyporesponsiveness to $1,25(OH)_2D_3$ may be an underlying contributing factor to the less positive calcium balance in HSLK-fed DS rats. Such a decrease in receptor sensitivity could, at least in part, account for the observed increases in renal and intestinal calcium losses.[7] Similar disturbances in calcium homeostasis and in $1,25(OH)_2D_3$ metabolism have also been reported in salt-fed prehypertensive DS rats,[16] suggesting that these alterations in calcium metabolism may be indigenous to this strain. Interestingly, our results also show that, compared with their DR counterparts, the DS rats have markedly reduced plasma levels of $25(OH)D_3$, the percursor of $1,25(OH)_2D_3$. The physiological significance, if any, of this difference in $25(OH)D_3$ cannot be determined from the present results. A time-dependent inverse relationship between $25(OH)D_3$ and ABP has recently been described by Thierry-Palmer *et al.* for DS rats on high dietary salt.[25]

We note that this relationship is not seen in spontaneously hypertensive rats,[25] suggesting that the salt-induced reduction in $25(OH)D_3$ may be specific to salt-sensitive variants of hypertension. More recently, the same authors reported that continuous delivery of exogenous $25(OH)D_3$ with subcutaneously implanted osmotic pumps failed to ameliorate the subsequent manifestation of hypertension in HS-fed, vitamin D-replete DS rats.[26] The cause for the salt-induced decrease in $25(OH)D_3$ in salt-sensitive rats is not known. It is likely that the increased biosynthesis of $1,25(OH)_2D_3$ in DS rats may contribute to the strain-dependent difference in $25(OH)D_3$ concentration. However, it seems unlikely that this could be the sole cause of the reduced $25(OH)D_3$ serum levels in DS rats given the almost 1000-fold higher plasma concentration of precursor compared with the concentration of biologically active hormone. Interestingly, the current results show that the strain-dependent difference in $25(OH)D_3$ is further exacerbated by

chemical sympathectomy with 6-OHDA, thereby suggesting a previously unrecognized element of regulation of $25(OH)D_3$ by the SNS. The potential for functional interactions between vitamin D and the SNS has previously been suggested by observations that vitamin D can upregulate catecholamine-synthesizing enzymes[19,20] and that vitamin D-dependent calcium-binding proteins can be localized in sympathetic nerve terminals.[15,17,18] However, a reciprocal interaction between sympathetic nerve function and vitamin D metabolism has not previously been demonstrated.

The question remains whether the observed differences in vitamin D metabolism and calcium handling between the DR and DS rats could contribute to the HSLK-induced differences in ABP between these strains. Dietary calcium supplementation exerts an antihypertensive effect[8] that is, at least partially, due to attenuation of sympathetic tone.[8,27] Because the current and previous[6,7] results show that sympathetic hyperactivity accounts fully for the hypertensive effect of HSLK in DR rats and, to a large extent, for the hypertensive effect of this diet in DS rats, it is conceivable that the HSLK-induced calcium loss may play a causative role in activating the SNS, both directly[27] and, possibly, by potentiating vitamin D-induced catecholamine synthesis.[19] In contrast, overactivation of the SNS, although maladaptive with respect to blood pressure homeostasis, may, indeed, be playing a beneficial role in calcium conservation, as discussed earlier. The anticalciuretic role of the SNS could, then, partly compensate for the HSLK-induced reduction in target organ responsiveness to $1,25(OH)_2D_3$ in DS rats. In contrast, the increase in plasma $1,25(OH)_2D_3$ could potentially contribute to hypertension development in DS rats. For example, receptors for $1,25(OH)_2D_3$ have been found in vascular smooth muscle[28] and both acute[29] and chronic[30] administration of the hormone raises peripheral vascular resistance and ABP, possibly by stimulating calcium influx via L-type channels.[31] Such vascular effects of $1,25(OH)_2D_3$ could, in principle, account for the sympathetic-independent component of HSLK-induced hypertension in DS rats.

In summary, the present study shows that DS rats maintained on a HSLK diet have higher $1,25(OH)_2D_3$ and lower $25(OH)D_3$ serum levels compared with DR rats and develop hypercalciuria independently of differences in renal sodium excretion. In conjunction with our previous observations[6,7] that DS rats maintained on this dietary regimen have less positive calcium balance due to increased excretory losses, the current findings suggest that a basic defect in calcium handling in DS rats may be hyporesponsiveness of renal and intestinal epithelium to the actions of the hormone. The increased levels of $1,25(OH)_2D_3$ may contribute to hypertension in DS rats by directly stimulating vascular smooth muscle contractility and sympathetic tone.

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