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DIETARY SODIUM INTAKE: INFLUENCE ON CALCIUM CHANNELS AND URINARY CALCIUM EXCRETION IN SPONTANEOUSLY HYPERTENSIVE RATS

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Abstract—Binding of the 1,4-dihydropyridine [^{3}H]PN200 110 was employed as an index of cardiac Ca^{2+} channels in normotensive (WKY) and spontaneously hypertensive (SHR) rats during 4 weeks of normal (0.73% NaCl) and high (8% NaCl) sodium diets when the rats were between 20 and 24 weeks of age. Binding site density was not different at the beginning of the study but was increased significantly ($P < 0.01$) after 1 week in the SHR on a high sodium diet; this difference was not apparent at 2, 3 or 4 weeks of the diet. During this same period, the urinary Ca^{2+} excretion in SHR was enhanced significantly ($P < 0.01$) and the urinary calcium/sodium ratio was elevated during the high sodium intake period.

Calcium channel antagonists, including the 1,4-dihydropyridines nifedipine and nitrendipine, are effective in reducing high blood pressure in hypertensive individuals [1–3]. Several observations indicate that sodium balance may modulate the efficacy of 1,4-dihydropyridine calcium antagonists. A high Na^+ intake may enhance the blood pressure lowering effects of nifedipine and other 1,4-dihydropyridines [4–6]. Moreover, 1,4-dihydropyridines exert a natriuretic effect both acutely [7–9] and after chronic administration [10, 11].

1,4-Dihydropyridines exhibit high-affinity specific binding to excitable tissues, including heart, and the determination of radioligand binding characteristics has proven to be very useful for the characterization of the L type of voltage-dependent Ca^{2+} channels [12, 13]. There is increasing evidence that these channels are regulated by both homologous and heterologous influences and according to pathologic state [14]. Thus, the numbers of Ca^{2+} channels may be altered in several animal models of hypertension in both heart and brain [15–20]. However, the relationship of these changes to the etiology of experimental hypertension remains to be established [14].

The level of dietary sodium intake is known to influence the activity of different receptors including cardiac beta-adrenoceptors, which are increased by high Na^+ intake [21]. Garthoff and Bellemann [22] reported that high salt intake and nitrendipine treatment could increase the number of nitrendipine sites in heart membranes from hypertensive rats.

The aim of this study was to investigate the influence of elevated dietary Na^+ intake on 1,4-dihydropyridine binding characteristics, as a measure of Ca^{2+} channels, in spontaneously hypertensive rats (SHR) with established hypertension and their normotensive Wistar Kyoto controls (WKY). We have included the analysis of urinary Ca^{2+} and Na^+ excretion in the normal and high Na^+ dietary protocols.

MATERIALS AND METHODS

Animal treatment. Thirty male normotensive Wistar Kyoto (WKY) and thirty male spontaneously hypertensive rats (SHR) of 20 weeks of age, after 1 week (baseline) of a normal Na^+ diet (0.73% NaCl), were divided in two subgroups: group 1 followed the same diet for an additional period of 4 weeks and group 2 followed a high Na^+ diet (8% NaCl) for an additional period of 4 weeks.

Blood pressure and body weight were measured at baseline and at the end of each diet period. Cardiac Ca^{2+} channels were assayed by (+)[^{3}H]PN200 110 binding at baseline and at weekly intervals. After 1 and 4 weeks of both Na^+ regimens urine was collected from rats placed overnight in metabolic cages without food, and urinary Na^+ and Ca^{2+} levels were measured.

Rats were obtained from Taconic (Germantown, NY). Blood pressures were measured by the tail cuff technique using a programmed electro-sphygmomanometer PE-300 (Narco Bio-Systems, Inc. Houston, TX). Urinary Ca^{2+} was determined by atomic absorption spectrophotometry and urinary Na^+ by flame photometry. Normal Na^+ (Agway Prolab 1000 with 0.73% NaCl) and high Na^+ diets (Agway Prolab 1000 with 8% NaCl) were obtained from ICN Biochemicals (Cleveland, OH).

Tissue preparation. After the initial 1 week (baseline), animals from the normal and high Na^+ subgroups of the WKY and SHR animals were killed at the rate of 3 per week. Heart ventricles were rapidly placed in ice-cold 50 mM Tris buffer (pH 7.2, 25°) and weighed. The ventricles were homogenized in 15 vol./g wet weight using a Tekmar Polytron at maximum setting for 5 sec followed by

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Table 1. Blood pressures and body weights of WKY and SHR at baseline and after 4 weeks of normal or high Na^+ diet

	Blood pressure (mm)		Body weight (g)	
	WKY	SHR*	WKY	SHR*
Baseline	126 ± 12	200 ± 5	441 ± 2	334 ± 2
Normal Na^+	139 ± 1	195 ± 4	498 ± 32	369 ± 2
High Na^+	126 ± 2	212 ± 12	445 ± 5	337 ± 4

Values are means ± SEM (baseline, N = 6; normal Na^+ and high Na^+, N = 3).
* Strain effect, F = 219, P < 0.001.

10 passes with a glass–Teflon homogenizer using a TRI-R STIR-R at a setting of 7.

[^{3}H]*PN*200 110 *binding*. The methods used were those described by Bolger *et al.* [23] and Janis *et al.* [24]. Heart ventricle homogenates were incubated with increasing concentrations of [^{3}H]PN200 110 at 25° for 150 min. This time was determined to be adequate for equilibration. After incubation, samples were filtered over Whatman GF/B filters and washed three times with 5 mL of ice-cold buffer using a cell harvester (model M24R, Brandel Instruments, Gaithersberg, MD). Radioactivity bound to filters was counted by liquid scintillation spectrometry at an efficiency of approximately 50%. Nonspecific binding was determined in the presence of 10^{-7} M (+)PN200 110. Initial control experiments established this incubation time as sufficient for equilibration to be reached.

Materials. (+)[^{3}H]PN200 110 [isopropyl-4-(2,1,3-benzoxadiazol) - dihydro - 5 - methoxycarbonyl - 2,6-dimethyl-3-pyridinecarboxylate]; sp. act. 85.9 Ci/mmol) was purchased from Dupont-New England Nuclear (Boston, MA). The enantiomers of PN200 110 were the gift of Dr. P. R. Hof (Sandoz, Basel, Switzerland). All other materials were obtained from commercial sources and were of the highest purity available.

Data analysis. Radioligand binding data were analyzed using a non-linear curve fitting program (BData, CData, EMF Software, Knoxville, TN) implemented on an IBM personal computer. Statistical analyses were performed by Student's *t*-test or analysis of variance (ANOVA) using an IBM personal computer and the Statistical Package for Social Sciences (SPSS v.3.0). All data are expressed as means ± SEM.

RESULTS

Body weight did not change significantly during the diet period in either WKY or SHR; however, SHR were lighter than WKY at all times studied (Table 1). Blood pressure (Table 1) was, as expected, significantly higher in SHR than in WKY; no significant changes in blood pressure were observed during either normal or high Na^+ intake in WKY or SHR. Significant left ventricular hypertrophy, expressed as percent of body weight, occurred in SHR throughout the study, but no differences were noted between normal and high Na^+ diets within each strain (SHR at baseline: 0.453 ± 0.016% of body weight, normal Na^+ diet: 0.407 ± 0.032, high Na^+ diet: 0.445 ± 0.005; WKY at baseline: 0.306 ± 0.020, normal Na^+ diet: 0.283 ± 0.07, high Na^+ diet: 0.306 ± 0.016; P < 0.001 SHR vs WKY).

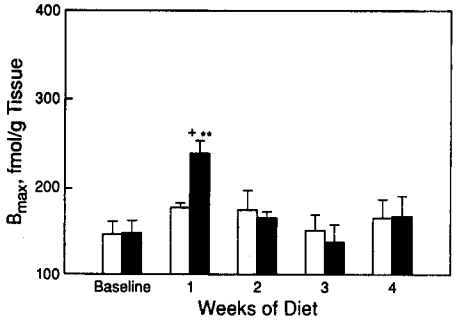

Fig. 1. 1,4-Dihydropyridine binding site density in cardiac preparations from SHR during normal (open bars) and high (filled bars) Na^+ diet. Key: (+) P < 0.05 vs baseline; and (**) P < 0.01 vs normal Na^+ diet. Data are expressed as means ± SEM (baseline, N = 6; weeks 1–4, N = 3).

The cardiac 1,4-dihydropyridine binding site density was not different in the two strains at the beginning of the study (WKY = 165 ± 9 fmol/g tissue, SHR = 146 ± 14 fmol/g tissue) and no significant changes were observed in binding density in WKY during the experimental period with either normal or high Na^+ intake. However, [^{3}H]PN200

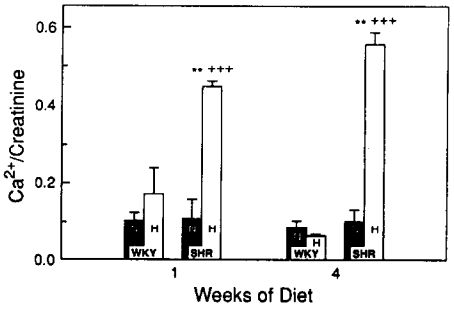

Fig. 2. Urinary Ca^{2+} excretion (expressed as the ratio between mmol of Ca^{2+} and mmol of creatinine excreted) in WKY and SHR during normal and high Na^+ diets. Key: (**) P < 0.01 SHR vs WKY; (+++) P < 0.001 high vs normal Na^+ diet. Data are expressed as means ± SEM (N = 3).

Table 2. Ratio between urinary Ca^{2+} excretion and urinary Na^+ excretion in WKY and SHR during both normal and high Na^+ diet

	Ca^{2+} excretion/Na^+ excretion			
	Normal Na^+ diet		High Na^+ diet	
	1 Week	4 Weeks	1 Week	4 Weeks
WKY	0.155 ± 0.075	0.166 ± 0.070	0.030 ± 0.008	0.043 ± 0.014
SHR	0.088 ± 0.036	0.099 ± 0.023	0.061 ± 0.001*	0.087 ± 0.004*

Values are means ± SEM (N = 3).
* $P < 0.05$ versus WKY within the same diet group.

110 maximum binding density (B_{max}) in hearts from SHR was increased significantly after 1 week of the high Na^+ diet to 245 ± 12 fmol/g tissue versus both controls (Fig. 1). [^{3}H]PN200 110 binding was of identically high affinity in both WKY and SHR at the beginning of the study (WKY = 5.4 ± 1.1 × 10^{-11} M, SHR = 5.4 ± 1.0 × 10^{-11} M), and no significant changes (range 3.0 to 7.5 × 10^{-11} M) were noted throughout the study.

Urinary Ca^{2+} excretion (expressed as the ratio between mmol of Ca^{2+} and mmol of creatinine excreted) (Fig. 2) was increased significantly in SHR during the high Na^+ diet after 1 week versus both SHR with normal Na^+ intake and WKY with the high Na^+ diet. After 4 weeks of diet the Ca^{2+} excretion remained significantly elevated in SHR. The urinary Ca^{2+}/Na^+ ratio (both Ca^{2+} and Na^+ were normalized for mmol of creatinine excreted) (Table 2) fell approximately 4- to 5-fold in WKY during the high Na^+ diet, while it remained unchanged in SHR during both Na^+ regimens and the difference between the two strains was statistically significant.

DISCUSSION

Previous reports indicate that Ca^{2+} channel antagonists are clinically more effective in lowering blood pressure during higher sodium intake [4, 5], and it has been suggested that this effect is not simply dependent on a greater degree of hypertension that occurs during the high Na^+ diet [6]. Garthoff and Bellemann [22] showed that both Na^+ intake and nitrendipine treatment increase the number of L type voltage-dependent Ca^{2+} channels present in heart cell membranes. Our results show an increase in cardiac Ca^{2+} channels, as indicated by 1,4-dihydropyridine binding, when the Na^+ content of diet was increased from 0.73% to 8%. However, this increase was limited to the first week of the diet and occurred only in SHR. Accordingly, the higher blood pressure lowering effect of 1,4-dihydropyridines, during the high Na^+ regimen, may be due to the natriuretic effect of these agents [7–11]. The lack of persistent up-regulation of Ca^{2+} channels in SHR during the high salt regimen may indicate the operation of a later counterregulatory process [14] and may constitute indirect evidence that these channels do not play a key role in the blood pressure increments caused by a high Na^+ diet in hypertensives. Hypercalciuria has been observed in several animal models of hypertension [25–28]; recent reports suggest that in Dahl-sensitive and in DOCA-salt hypertensive rats urinary Ca^{2+} excretion is related to dietary NaCl [29, 30]. Our study shows that during the high Na^+ diet there was enhanced Ca^{2+} excretion in SHR relative to WKY so that the Ca^{2+}/Na^+ ratio became significantly higher in SHR during high Na^+ intake. Since urinary Ca^{2+} remained altered after 4 weeks of diet whereas Ca^{2+} channel density was elevated for only the initial week, these two events may not be linked. The analysis of the Ca^{2+}/Na^+ ratio supports the hypothesis that the altered renal Ca^{2+} handling is, at least in part, Na^+ dependent.

In summary, this study shows that an elevated dietary Na^+ uptake was associated with a temporary increase of 1,4-dihydropyridine binding sites in hearts from SHR with established hypertension but not from WKY. However, during this same dietary period there was a persistent elevation of urinary Ca^{2+} excretion likely coupled to enhanced Na^+ excretion in SHR.

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REFERENCES

1. Aoky K, Kando S and Mochizuki A, Antihypertensive effects of cardiovascular-antagonist in hypertensive patients in absence of beta-adrenergic blockade. *Am Heart J* **96**: 218–226, 1978.
2. Bühler FR, Hulten VL and Bolli P, Calcium channel inhibitors for identification of mechanisms and treatment of hypertension. *J Cardiovasc Pharmacol* **4**: S267–S268, 1982.
3. Ferrara Fasano ML, de Simone G, Soro S and Gagliardi R, Antihypertensive and cardiovascular effects of nitrendipine: A controlled study vs placebo. *Clin Pharmacol Ther* **38**: 435–438, 1985.
4. MacGregor GA, Cappuccio FP and Markandu ND, Sodium intake, high blood pressure and calcium channel blockers. *Am J Med* **82**: (Suppl 3B): 16–22, 1987.
5. Cappuccio FP, Markandu ND and MacGregor GA, Calcium antagonists and sodium balance: Effects of changes on sodium intake and of the addition of

thiazide diuretics on the blood pressure lowering effect of nifedipine. *J Cardiovasc Pharmacol* **10** (Suppl 10): S57–S60, 1987.
6. Galletti F, Strazzullo P, Cappuccio FP, Barba G, Girgione N, Gagliardi R and Mancini M, Calcium-channel blockers and sodium intake: A controlled study in patients with essential hypertension. *Cardiovasc Drugs Ther* **3**: 135–140, 1989.
7. Zanchetti A, Stella A and Golin R, Adrenergic sodium handling and natriuretic action of calcium antagonists. *J Cardiovasc Pharmacol* **7** (Suppl 6): S194–S198, 1985.
8. Ene MD, Williamson PS, Roberts CJC and Waddel G, The natriuresis following oral administration of the calcium antagonists nifedipine and nitrendipine. *Br J Clin Pharmacol* **19**: 423–427, 1985.
9. Krussel LR, Christensen CK and Lederballe PO, Acute natriuretic effect of nifedipine in hypertensive patients and normotensive controls. A proximal tubular effect? *Eur J Clin Pharmacol* **32**: 121–126, 1987.
10. Ruilope LM, Miranda B, Oliet A, Alcazar J, Bigorra J and Rodicio JL, Persistence of the natriuretic effect of calcium entry blockers. *J Cardiovasc Pharmacol* **12** (Suppl 4): S136–S139, 1985.
11. Nicholson JP, Resnik LM and Laragh JH, Hydrochlorothiazide is not additive to verapamil in treating essential hypertension. *Arch Intern Med* **149**: 125–128, 1989.
12. Janis RA, Silver PJ and Triggle DJ, Drug action and cellular calcium regulation. *Adv Drug Res* **16**: 309–591, 1987.
13. Triggle DJ, Langs DA and Janis RA, Ca^{2+} channel ligands: Structure–function relationship of the 1,4-dihydropyridines. *Med Res Rev* **9**: 123–180, 1989.
14. Ferrante J and Triggle DJ, Drug and disease-induced regulation of voltage-dependent calcium channels. *Pharmacol Rev* **42**: 29–44, 1990.
15. Ishii K, Kano T, Kurobe Y and Ando J, Binding of [^{3}H]nitrendipine to heart and brain membranes from normotensive and SHR. *Eur J Pharmacol* **88**: 277–278, 1983.
16. Chatelain P, Demol D and Roba J, Comparison of [^{3}H]nitrendipine binding to heart membranes of normotensive and spontaneously hypertensive rats. *J Cardiovasc Pharmacol* **6**: 220–223, 1984.
17. Dillon JS, Gu XH and Nayler WG, Effect of age and of hypertrophy on cardiac Ca^{2+} antagonist binding sites. *J Cardiovasc Pharmacol* **14**: 233–240, 1989.
18. Lee HR, Watson M, Yamamura HI and Roeske WR, Decreased [^{3}H]nitrendipine binding in the brainstem of deoxycorticosterone-NaCl hypertensive rats. *Life Sci* **37**: 971–977, 1985.
19. Ross D and Morris N, Regional and subcellular distribution of [^{3}H]nitrendipine binding sites in SHR and WKY rats. *J Cardiovasc Pharmacol* **12** (Suppl 4): S94–S97, 1988.
20. Huguet F, Huchet A-M, Gerard P and Narcisse G, Characterization of dihydropyridine binding sites in the rat brain: Hypertension and age-dependent modulation of [^{3}H](+)-PN 200-110 binding. *Brain Res* **412**: 125–130, 1987.
21. Limas C and Limas CJ, Cardiac beta-adrenergic receptors in salt-dependent genetic hypertension. *Hypertension* **7**: 760–766, 1985.
22. Garthoff B and Bellemann P, Effects of salt loading and nitrendipine on dihydropyridine receptors in hypertensive rats. *J Cardiovasc Pharmacol* **10** (Suppl 10): S36–S38, 1987.
23. Bolger GT, Gengo R, Klochowski R, Luchowski E, Siegel H, Janis RA, Triggle AM and Triggle DJ, Characterization of binding of the Ca^{2+} channel antagonist, [^{3}H]nitrendipine, to guinea pig ileal smooth muscle. *J Pharmacol Exp Ther* **225**: 291–301, 1983.
24. Janis RA, Sarmiento JG, Maurer SC, Bolger GT and Triggle DJ, Characteristics of the binding of [^{3}H]nitrendipine to rabbit ventricular membranes: Modification by other Ca^{2+} channel antagonists and by the Ca^{2+} channel agonist Bay K 8644. *J Pharmacol Exp Ther* **231**: 8–15, 1984.
25. McCarron DA, Yung NN, Ugoretz BA and Krutzik S, Disturbances of calcium metabolism in the spontaneously hypertensive rat. *Hypertension* **3** (Suppl 1): I162–I167, 1981.
26. Lau K, Zikos D, Spirnak J and Eby B, Evidence for an intestinal mechanism in hypercalciuria of spontaneously hypertensive rats. *Am J Physiol* **247**: E625–E633, 1984.
27. Cirillo M, Galletti F, Corrado MF and Strazzullo P, Disturbances of renal and erythrocyte calcium handling in rats of the Milan hypertensive strain. *J Hypertens* **4**: 443–449, 1986.
28. Cirillo M, Galletti F, Strazzullo P, Torielli L and Melloni MC, On the pathogenic mechanism of hypercalciuria in genetically hypertensive rats of the Milan strain. *Am J Hypertens* **2**: 741–746, 1989.
29. Kurtz TW and Morris RC, Dietary chloride as a determinant of disordered calcium metabolism in salt dependent hypertension. *Life Sci* **36**: 921–928, 1985.
30. Kotchen TA, Ott CE, Whitescarver SA, Resnick LM, Gertner JM and Blehschmidt NG, Calcium and calcium regulating hormones in "prehypertensive" Dahl salt-sensitive rats. *Am J Hypertens* **2**: 747–753, 1989.