Effect of calcium on blood pressure, platelet aggregation and erythrocyte sodium transport in Dahl salt-sensitive rats

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Forty 11-week-old Dahl salt-sensitive rats were divided into four groups matched for blood pressure and weight. Group I was given a sodium-deficient diet, group II a sodium-enriched diet, group III a sodium-enriched diet plus a calcium supplement and group IV a sodium-enriched diet plus nitrendipine, a calcium antagonist. For the first 18 weeks, when the sodium-enriched diet contained 2.6% sodium, there were no differences in blood pressure between the groups; the sodium content was then increased to 8%, and the diets continued for 12 more weeks. At 41 weeks old, the rats in group II had significantly($p<0.05$) higher systolic blood pressures than the other groups. Erythrocytes from the rats on the low-sodium diet had significantly($p<0.025$) lower intracellular sodium(3.9 ± 0.4mmol/l) while cells from the rats given nitrendipine had significantly($p<0.005$) higher intracellular sodium(13.3 ± 0.8mmol/l) than those from the rats on a high-salt diet(7.4 ± 1.4mmol/l). Nitrendipine caused significant ($p<0.05$) decreases in both ouabain-sensitive and furosemide-sensitive sodium efflux. Platelet aggregation in response to 2 μmol/l adenosine diphosphate was not significantly affected by the nitrendipine. The evidence that nitrendipine markedly affects sodium transport supports the hypothesis that an interaction of calcium and sodium may be involved in blood pressure control.

Journal of Hypertension 1990, 8:245-250

Keywords: Nitrendipine, sodium pump, intracellualr sodium.

Introduction

The possible role of calcium in the pathogenesis and treatment of Hypertension has recently received much attention [1, 2]. Many studies, both in humans [3-11] and in rats [12-16], provide evidence for an inverse relationship between dietary calcium and blood pressure. Dietary sodium, However, apperars to have a direct relationship with hypertension. Its role in the development of hypertension and the effectiveness of dietary sodium restriction in treating hypertension were first suggested in 1904 [17] and have been amply supported through the

years [18-22]. The debate about the relative importance of sodium and calcium [1, 23] has implied an 'either/or' hypothesis that may be diverting attention away from a possible interaction between calcium and sodium in the etilolgy of hypertension. Blaustein and Hamlyn [24] have proposed that the underlying factor may be impaired renal sodium excretion, possibly exacerbated by increased dietary sodium. The resultant increase in sodium and water may be countered by the synthesis of a sodium-pump inhibitor [25, 26]. Although this inhibitor would decrease renal sodium reabsorption, it could increase intracellular sodium concentrations in other tissues by preventing sodium efflux. The increased intracellular sodium could then promote sodium-calcium exchange, leading to higher intracellular calcium concentrations that might be responsible for increased vascular reactivity and thus hypertension. Platelet activity may reflect cellular calcium handling since aggregation is calcium-dependent [27], apparently requiring a calcium influx. Erne et al. [28] have reported that levels of platelet intracellular calcium are directly correlated with blood pressure.

The Dahl salt-sensitive (Dahl S) rat, an animal that becomes Hypertensive when given a high-salt diet, is an appropriate model for examining the possible interaction between sodium and calcium. We compared blood pressure, platelet aggregation and sodium transport in erythrocytes of Dahl S rats on a sodium-deficient diet with those of rats on a high-salt diet. We also examined the effect of altering calcium status by supplementing the high-salt diet with calcium or nitrendipine, a calcium antagonist.

Methods

Animals and diets

Forty 3-week-old male Dahl S rats (Brookhaven, Upton, New York, USA) were fed freely on an AIN 76A semipurified diet(0.4 % sodium) for 8 week to establish base-line systolic blood pressures. The rats were then dividied into four groups of 10 matched for body weight and systolic blood pressure, and fed the following diets: group Ⅰ: AIN 76A semipurified sodium-deficient diet (〈0.1% sodium; ICN Biochemicals, Cleveland, Ohio, USA); group Ⅱ: AIN 76A diet with NaCl(2.6%, then 8% sodium); group Ⅲ: AIN 76A diet with NaCl(2.5%, then 8% Sodium) and $CaCO_3$ (2% calcium); group Ⅳ: AIN 76A diet with NaCl (2.6%, then 8% sodium) and 2000 ppm nitrendipine(Miles Pharmaceuticals, New Haven, Connecticut, USA) added to the diet.

The rats were individually housed in stainless -steel cages in a temperature and humidity controlled environment with a daily 12-h light and 12-h dark cycle. Distilled water was freely available. When it became evident, after 18 weeks, that 2.6% sodium was not sufficient to cause hypertension, the sodium content was increased to 8% and the diets were continued until termination at 42-44 weeks of age. Because rats are nocturnal and nitrendipine is

light-sensitive, the diets were replenished within 30 min of the lights being turned off each day to ensure that the nitrendipine was consumed while it was active. Food intake was measured daily, weight gain was measured weekly and systolic blood pressure was monitored every 2 weeks using a Harvard Bioscience Model 52-0338-0 tailcuff apparatus (South Natick, Massachusetts, USA) with a Macrobiosystem MK-Ⅲ Physiograph (Houston, Texas, USA). This apparatus can be used with awake rats and does not require heating. Three reading for each rat were averaged.

Platelet aggregation

At the age 42-44 weeks, the rats were anesthetized with an intramuscular injection of a mixture of ketamine hydrochloride and xylazine (90: 10mg/kg body weight) and blood was collected into heparinized vacutainer tubes via cardiac puncture. Platelet aggregation was measured in whole blood using a Chronolog Model 500 Whole Blood Aggregometer (Havertown, Pennsylvania, USA). Concentration of approximately 200,000 platelets/μL Adenosine diphosphate(ADP; 2mol/l) was added to initiate aggregation, and three readings of impedance changes were averaged for each rat. This concentration of ADP was chosen because it gave measurable aggregation without causing a saturated response in rat whole blood. The impedance was quantitated by comparison with the recorder response to a 5Ω impedance. The impedance method of determining platelet aggregation in whole blood was introduced in 1980 by Cardinal and Flower [29] using human blood. Platelet aggregation causes an increase in impedance between two platinum wires placed in the blood. Our data indicate that platelet aggregation determined by the impedance method in whole blood correlates significantly($r=0.74$, $p<0.001$, $n=44$) with aggregation determined by the turbidometric method in platelet-rich plasma. The impedance method has the advantage of measuring platelet aggregation under nearly physiological conditions with the aggregation performed within minutes of venipuncture and in the presence of other blood components.

Erythrocyte studies

Blood collected in heparinized vacutainer tubes was centrifuged at 1000g for 10min, and the plasma and buffy coat were removed. The sodium efflux determinations followed previously published procedures [30-32]. The erythrocytes were washed six times with a cold isotonic washing solution [150mol/l choline chloride, 10mol/l TRIS-4-morpholinopropane sulfonic acid(MOPS), pH 7.4 at 4℃]. Approximately five times the erythrocyte volume was used for each wash, centrifuging at 1000g for 5min after each wash. The final erythrocyte pellet was suspended in the choline chloride solution to give 40-50% hematocrit. The hematocrit was measured. A 50-μl aliquot of the suspended erythrocytes was added to 5ml of 0.02% acationox (a metal-free detergent, Scientific Products, McGaw Park, Illinois, USA) to be used for de-

termination of intracellular sodium and potassium concentrations.

Sodium efflux from erythrocytes occurs by at least three different pathways, the sodium pump, sodium-potassium cotransport and sodium leaks. Sodium efflux through the pump can be measured as the ouabain-inhibitable efflux into a $MgCl_2$ medium. Sodium-potassium cotransport can be measured as the furosemide (Hoechst-Roussek Pharmaceuticals, Sommerville, New Jersey, USA) - inhibitable efflux into a choline chloride medium. The leak is the efflux that is not inhibited by either ouabain or furosemide. The following solutions were used as efflux media: medium 1: 70mmol/l $MgCl_2$, 10mmol/l KCl, 85mmol/l sucrose, 10mmol/l glucose, 10mmol/l TRIS-MOPS, pH 7.4 at 37℃; medium 2: medium 1 plus 1 mmol/l ouabain; medium 3: 150mmol/l choline chloride, 10mmol /l glucose, 1mmol/l ouabain, 10mmol/l TRIS-MOPS, pH 7.4 at 37℃; medium 4: medium 3 plus 1mmol/l furosemide.

Four millilitres of the erythrocyte suspension were added to 50ml medium 1 and 40ml medium 2 for determination of sodium efflux via the sodium pump. Two millilitres of the erthrocyte suspension were added to 40ml medium 3 and to 40ml medium 4 for determination of sodium-potassium cotransport. The erythrocytes in each medium were mixed and aliquoted into 12 tubes, and transferred to a shaking water bath at 37 ℃. Duplicate tubes containing media 1 and 2 were removed and placed in an ice bath for 0, 2, 4, 6, 8 and 10min. Duplicate tubes containing media 3 and 4 were removed after 0, 10, 20, 30, 40 and 50min. Tubes were Centrifuged at 1, 000g for 5 min, the Supernatants removed and stored, capped and refrigerated until the sodium concentration was measured.

Calculations

A sample calculation showing the conversion of sodium efflux expressed as μg/ml per min to mmol/l erythrocytes per h is given below. In this determination, 4ml erythrocyte suspension was added to 40ml incubation medium.

$$\mu g/(ml \times min) \times 60min \times mmol/23mg \times [44ml - (4ml \times hct)/(0.0041 \times hct)] \times (mg/1000\mu g)$$

$$= mmol/l \text{ erythrocytes per h}$$

Sodium efflux via the sodium pump was calculated as the difference between the efflux into medium 1 and that into medium 2; sodium-potassium cotransport was calculated as the difference between the efflux into medium 3 and that into medium 4. The leak was calculated as the efflux into medium 4.

Fifty microlitres of the erythrocyte suspension were diluted with 5ml 0.02% acationonx for determination of the intracellular sodium concentration. Two hundred microlitres of the sodium dilution were further diluted with 2ml deionized water for determination of the intracellular potassium concentration. These concentrations of intracellular sodium and potassium were also the concentrations in the cells during the efflux determinations.

Plasma concentrations of calcium, sodium and potassium

The plasma calcium concentration was determined on a sample prepared by diluting 1ml plasma with 9ml of a solution of lanthanum chloride(17.8g/1) and trichloracetic acid(40g/1). The sample was mixed, allowed to stand 10 min and centrifuged at 1000g for 10min. The calcium concentration of the supernatant was measured, and the plasma potassium concentration was determined in a 1 : 100 dilution of plasma in deionized water; the plasma sodium concentration was determined in a 1 : 400 dilution of plasma in water.

All sodium, potassium and calcium concentrations were measured with a Perkin-Elmer Atomic Absorption Spectrophotometer, model 373(Norwalk, Connecticut, USA).

Plasma nitrendipine concentrations were determined by radio-immunoassay using an antidihydropyridine antibody supplied by Dr Kevin Campbell of the University of Iowa [33, 34]

Results

Throughout the study, the rats in all groups appeared healthy and gained weight normally. There were no significant differences among the weights of the rats in the four diet groups. The systolic blood pressures of the rats consuming 2.6% sodium were not significantly different from those on the sodium-deficient diet. After 18 weeks of 2.6% sodium-supplemented diets, the sodium was increased to 8.0% for the rats in groups Ⅱ-Ⅳ. After 10 weeks on the 8.0 % sodium diets, the rats ingesting neither the calcium supplement nor nitredipine had significantly($p<0.05$) higher systolic blood pressure than the other three groups(172.0 ± 9.8 versus 155.3 ± 10.0mmHg, Fig. 1).

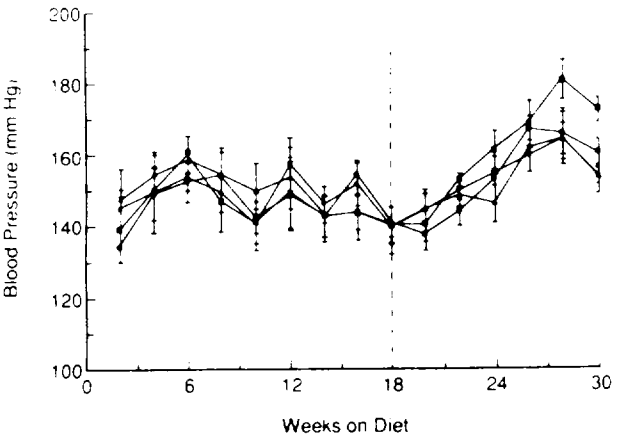

Fig. 1. Effect of diet on systolic blood pressure. ▲—▲, sodium-deficient diet; ■—■, high-sodium diet, ●—●, high-sodium plus calcium diet; ◆—◆ high-sodium plus nitrendipine diet. The dotted line marks the change in the high-sodium diets from 2.6% to 8.0% sodium

Platelet aggregation was measured at the end of experimental period after 30 weeks on the special diets. There were no significant differences in maximum aggregation between any two groups(Table 1). When the rats on low sodium were compared with all($n=26$) of the rats on high sodium, the platelet aggregation was significantly($p<0.01$) lower. For the rats on the low-salt diet, the initial slope of the aggregation (Ω/min) was significantly($p<0.01$) less than for the rats on the high-salt diets but there was no indication of disaggregation after 6 min, while there was maximum aggregaion in the platelets from the rats on high-salt diets after 3-4min.

Table 1. Platelet aggregation in Dahl salt-sensitive rats.

	Diets			
	Low sodium (n = 10)	High sodium (n = 7)	High sodium + calcium (n = 9)	High sodium + ND (n = 10)
Aggregation (Ω)	16.5 ± 2.5*	18.7 ± 2.5	18.5 ± 1.2	18.7 ± 2.5
Initial slope (Ω / per min)	33.8 ± 4.5**	45.9 ± 5.8	43.3 ± 4.5	45.7 ± 7.3

Means ± s.d. ND, nitrendipine. *$P < 0.01$, **$P < 0.001$, versus all other groups.

Table 2 shows the values for plasma calcium, plasma sodium, and plasma potassium, none of which was significantly different among the groups. The intracellular sodium concentration was significantly($p<0.025$) lower in the rats on the sodium-deficient diets and significantly($p<0.05$) higher in the rats ingesting nitrendipine than the rats on the higher in the rats ingesting diet with no supplements. Intracellular potassium was higher($p<0.05$) in the rats ingesting nitrendipine than in the other three groups.

Table 2. Plasma and intracellular ion concentrations (mmol/l) in Dahl salt-sensitive rats

	Diets			
	Low sodium	High sodium	High sodium + calcium	High sodium + ND
Plasma Ca^{2+}	2.5 ± 0.1 (10)	2.5 ± 0.1 (6)	2.5 ± 0.2 (8)	2.4 ± 0.1 (10)
Plasma Na^+	116 ± 4 (10)	123 ± 8 (6)	120 ± 6 (8)	118 ± 5 (10)
Plasma K^+	4.8 ± 0.4 (10)	4.5 ± 0.6 (6)	4.7 ± 0.3 (8)	4.7 ± 0.3 (10)
Erythrocyte Na^+	3.9 ± 1.1** (10)	7.4 ± 3.7 (7)	9.4 ± 4.8 (9)	13.3 ± 2.4*** (10)
Erythrocyte K^+	87 ± 16 (10)	93 ± 2 (7)	90 ± 9 (8)	101 ± 10* (10)

Means ± s.d. ND, nitrendipine. Number of rats per group in parentheses. *$P < 0.05$, **$P < 0.025$, ***$P < 0.005$, versus high-sodium diet

The results for the erythrocyte sodium transport measurements are shown in Fig. 2. In the rats on the 8.0% sodium-supplemented diets plus nitrendipine there was significantly less sodium efflux via the ouabain-sensitive sodium pump($p<0.05$) and significantly less furosemide-inhibitable sodium-potassium cotransport($p<0.05$) than the other three groups. There was also a significant difference($p<0.05$) between the sodium efflux via the leak between the rats on the nitrendipine-supplemented and calcium-supplemented diets.

The mean plasma nitrendipine concentration for the rats on nitrendipine was $5.1 \pm 1.6 \mu$mol/l. The values for the other rats were zero.

Discussion

Previous studies of Dahl S rats have typically used an 8.0% sodium diet to cause hypertension. In our study, a diet with moderately elevated sodium(2.6%) did not cause a significant elevation in blood pressure. The normal AIN 76A diet contains 0.1% sodium [35]. Neither calcium supplements nor nitrendipine had a noticeable effect on the blood pressure of the rats on the 2.6% sodium diets. The blood pressure lowering effects of the calcium and nitrendipine were evident only for rats consuming 8.0% sodium. These results support the observation of Resnick et al. [36] that calcium lowers the blood pressure of human subjects only when they are consuming high-salt diets,

and also agree with the observation of

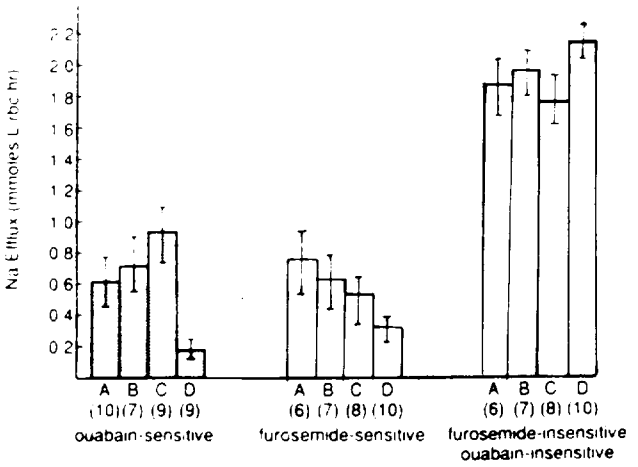

Fig. 2. Effect of diet on erythrocyte sodium efflux. A, sodium-deficient diet; B, high-sodium diet; C, high-sodium plus calcium diet; D, high-sodium plus nitrendipine diet; rbc, erythrocytes.

McCarron et al. [37] that an elevated salt diet is necessary for calcium to have a hypotensive effect.

The effect of nitrendipine on Dahl S rats on high-salt diets differs from the reported effects of nitrendipine in black hypertensive subjects [38]. Although the blood pressure was lowered in both the Dahl S rats and the black hypertensives, the rats had increased intracellular sodium and decreased sodium pump activity; the black hypertensives showed no changes in intracellular sodium or sodium pump activity. One reason for the difference may be that the black subjects were on moderately low-sodium diets(92 ± 10mmol/24h) while the rats were on elevated-sodium diets. These differences indicatd that Dahl S rats may not be a good model for hypertensive black subjects.

The effect of nitrendipine on intracellular sodium and the ouabain-sensitive sodium efflux are in contrast to the effects of nifedipine, another calcium antagonist, reported by Knorr et al. [39]. They observed that nifedipine caused a decrease in intracellular sodium and an increase in the sodium pump activity of erythrocytes from Dahl S rats that began consuming 8% sodium diets at 4 weeks of age. They also observed that the sodium pump was completely suppressed in erythrocytes from salt-loaded Dahl S rats; the sodium pump of the rats on high-salt diets in our study was not significantly altered.

In the present study the decrease in the sodium pump activity of the erythrocytes from rats consuming nitrendipine is also in contrast with data reported from the Panand Janis study [40] on the effect of nitrendipine on Na^+, K^+ - ATPase from several membranes.

The absence of a nitrendipine effect on platelet aggregaion was surprising. Other calcium antagonists such as nifedipine, verapamil and diltiazem inhibit platelet aggregation [41-45], both when administered to the subject and when added to the platelet-rich plasma. It is possible that the measurement of platelet aggregation in whole blood is not so sensitive to nitrendipine as it is in platelet rich plasma. In our study, the measurable plasma concentrations of nitrendipine confirmed that the drug had been absorbed and was present in the plasma at the time the platelet aggregaion experiments were conducted.

The data in this study suggest that the mechanisms through which calcium supplements and

nitrendipine exert their hypotensive effects are probably very different. Both the calcium-supplemented group and the nitrendipine group showed elevated intracellular sodium, but the ouabain-inhibitable sodium efflux was increased in the calcium-supplemented group and decreased in the nitrendipine group. The combination of decreased sodium pump activity and an increased intracellular sodium concentration in the nitrendipine group suggests that nitrendipine may be acting as an inhibitor of the sodium pump. The observation that both calcium supplements and nitrendipine affect the intracellular sodium concentrations and sodium pump activities of the Dahl S rats on high-salt-diets supports the hypothesis that an inter action of calcium and sodium may be involved in blood pressure control.

Acknowledgements

The nitrendipine was a gift from Miles Laboratories(New Haven, Connecticut, USA) and the furosemide was a gift from Hoechst-Roussel Pharmaceuticals (Somerville, New Jersey, USA). We also thank Drs Campbell and Kahl for the nitrendipine antibody and directions for the radio-immunoassay, and Mrs Dawn Haan for secretarial assistance.

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