Effect of Sodium Intake on the Hypotensive Effect of Calcium Antagonists

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SUMMARY

To clarify the influence of Na balance on the hypotensive effect of calcium antagonists, the changes of blood pressure and humoral factors after a single oral administration of 40 mg nicardipine were evaluated in 15 subjects with essential hypertension under high, normal, and low Na regimens (mean 24 hour urinary Na excretion: 320±24, 147±7, 27±6 mEq, respectively). Nicardipine induced a significant reduction of mean blood pressure and increase in heart rate. The change of mean blood pressure after nicardipine was negatively related to the pretreatment mean blood pressure under the three levels of Na intake (p<0.01). The slopes of the correlation lines for high, normal, and low Na regimens were -0.61, -0.69, and -0.52, respectively, without statistical significance. Nicardipine brought about significant increases in plasma renin activity and plasma norepinephrine, but no changes in plasma levels of epinephrine, 6-keto-prostaglandin F1α, thromboxane B2 or serum aldosterone concentration. These results suggest that the magnitude of the untreated blood pressure and thereby the peripheral resistance are major determinants of the blood pressure fall caused by calcium antagonists, and that the failure to increase aldosterone and epinephrine in the face of peripheral vasodilation may be responsible in part for the hypotensive effect of this drug.

Additional Indexing Words:
Calcium antagonist Sodium intake Hypertension Aldosterone Catecholamines Prostaglandins

CALCIUM (Ca)-antagonists inhibit the cellular transmembrane influx of Ca++ and the release of Ca++ from intracellular storage sites in the sarcolemmal membrane or sarcoplasmic reticulum of vascular smooth muscle cells by selective inhibition of the Ca-channel on the cell membrane which results in the relaxation of vascular smooth muscle.1,2) Ca-antagonists have
been used as vaso-relaxing agents for the treatment of angiospastic diseases such as angina pectoris, hypertension, Raynaud’s phenomenon and cerebral vasospasm. Concerning the efficacy of Ca-antagonists in the treatment of essential hypertension, it has been proposed that it may be possible to use Ca-antagonists as a first-step regimen in the stepped-care approach to drug therapy, which has been recommended by the U.S. Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure. Previous reports, in which factors responsible for the hypotensive effect of Ca-antagonists have been evaluated in patients with essential hypertensions, have indicated that the effect of Ca-antagonists is modulated by the pretreatment blood pressure, age, plasma renin activity (PRA) and/or Na balance.

In this study, we examined the changes of blood pressure, the renin-angiotensin-aldosterone system, plasma catecholamines and prostaglandins after a single oral administration of the Ca-antagonist nicardipine in subjects with essential hypertension who were placed on high, normal and low Na regimens for the purpose of clarifying the influence of Na balance on the hypotensive effect of Ca-antagonists.

METHODS

The subjects were 15 patients (9 men and 6 women; age range 36 to 60 years; mean age 49 years) with essential hypertension. Diagnosis of essential hypertension was made by physical examination, routine laboratory examinations, and endocrinologic and radiologic studies. Outpatients with a blood pressure over 160 mmHg systolic or 95 mmHg diastolic and without a history of taking antihypertensives were selected for the study. Our subjects included 10 cases at stage I and 5 cases at stage II according to the WHO classification.

After hospitalization, all subjects were taking a normal Na diet in which 10 g per day of salt was used for cooking for at least 7 days, to stabilize blood pressure and Na balance. The control period when the subjects were taking the normal Na diet was followed by 5 days of Na loading and by 5 days of Na restriction. During Na loading the patient received a salt load of 27 g per day with high Na diet in which 18 g per day of salt was used for cooking supplemented with 15 tablets of Slow Na (Ciba-Geigy Ltd., Basel, Switzerland, 600 mg of salt per tablet). Na restriction was done by placing the subjects on a low Na diet in which no salt was used for cooking. Each Na diet contained 1.7 g of K and 60 g of protein. The subjects were instructed to avoid any food intake other than hospital diet and to refrain from smoking during the study. In the early morning of the 6th day of the individual Na
regimens, changes of blood pressure, heart rate and plasma humoral factors after a single oral administration of nicardipine were determined. Twenty-four hour urinary excretion of Na and creatinine the day before examination was determined to check the Na balance.

Experiments were carried out in the morning with the patient fasting and resting in a supine position after voiding urine and having body weight measured. A venous catheter was introduced into the antecubital vein and the Automated System for Measuring Blood Pressure (BP-203, Nihon-Cohrin Co., Tokyo) was placed on the other arm for measurements of blood pressure and heart rate. Thirty minutes after implantation of the catheter, blood samples were taken from the venous catheter for the determinations of PRA, serum aldosterone (Ald), plasma levels of norepinephrine (PNE), epinephrine (PE), thromboxane B₂ (TX B₂) and 6-keto-prostaglandin F₁α (6-keto-PG F₁α). The averages of blood pressure and heart rate measured 5 times at intervals of 3 min after the blood sampling were used for the pretreatment values of blood pressure and heart rate. After the baseline data were obtained, the subject took 40 mg of nicardipine orally, and the blood pressure and heart rate were monitored every 5 min. In most subjects, nicardipine showed its maximum hypotensive effect during 60 to 80 min after the oral administration of the drug. The same measurements as in the control period were then performed 70 min after each subject had taken nicardipine.

The blood specimens for PRA, PNE and PE were placed in chilled tubes containing disodium ethylenediamine tetraacetate (EDTA-Na₂) and immediately centrifuged at 3,000 rpm for 30 min (4°C). PRA was determined by the radioimmunoassay of generated angiotensin I,¹¹ using a Gamma Coat (¹²¹I) PRA Radioimmunoassay Kit (Baxter Healthcare Co., Cambridge, Massachusetts). PNE and PE were determined according to the modified trihydroxyindole method of Anton and Sayre,¹² using high pressure liquid chromatography.¹³ The details of this method have been described elsewhere.¹⁴

The blood specimens for measurements of TX B₂ and 6-keto-PG F₁α were placed in chilled tubes containing EDTA-Na₂ (2 mg/ml) and aspirin (0.5 mg/ml) and centrifuged at 3,000 rpm for 30 min. Prostaglandins in the plasma were extracted by the method of Jaffe et al.¹⁵ A 1 ml sample of plasma was mixed with 3 ml of petroleum ether to remove neutral lipids. The aqueous layer was exposed to 3 ml of 1:1 ethyl acetate: isopropanol, and vortexed, and a mixture of 2 ml of ethyl acetate and 3 ml of water was added. After further mixing, the two phases were separated by centrifugation. After the organic phase was evaporated dry in N₂ gas at 55°C, it was dissolved in 1 ml of a mixture of benzene, ethyl acetate and methanol (60:40:2, v/v).
The dissolved material was applied to a silicic acid column and the fractions of TX \(B_2\) and 6-keto-PG \(F_{1\alpha}\) were obtained by developing the column serially with the eluent consisting of benzene: ethyl acetate: methanol. Prostaglandin concentrations in each fraction extracted from plasma were radioimmunoassayed by the double-antibody procedure of Morris et al.\(^{16}\) utilizing highly specific antisera for TX \(B_2\) and 6-keto-PG \(F_{1\alpha}\) purchased from Ono Pharmaceutical Co. (Osaka, Japan). The cross-reactivity of the antiserum of TX \(B_2\) was <0.1% with PG \(E_1\), PG \(E_2\), PG \(F_{1\alpha}\), PG \(F_2\alpha\) and 6-keto-PG \(F_{1\alpha}\). The cross-reactivity of the antiserum of 6-keto-PG \(F_{1\alpha}\) was 2.0% with PG \(E_1\), 8.4% with PG \(E_2\), 4% with PG \(F_2\alpha\), <0.1% with PG \(F_{1\alpha}\) and TX \(B_2\). Intra-assay variations were 3.6% for TX \(B_2\) and 10.5% for 6-keto-PG \(F_{1\alpha}\). Interassay variations were 8.4% for TX \(B_2\) and 13.7% for 6-keto-PG \(F_{1\alpha}\). Ald was measured by a non-chromatographic non-extraction radioimmunoassay method\(^{17}\) with an Aldosterone RIA Kit (Abbott Lab., Chicago, Illinois). Urinary Na and creatinine were measured by an Autoanalyzer ASTRA-4 (Beckman Instruments, Los Angeles, California).

Measured variables are expressed as mean±standard error of the mean (SEM). The Student’s paired t-test was used for the examination of within-group changes.

**Results**

Changes of blood pressure, heart rate, body weight and endocrinologic findings on three Na regimens are shown in Table I. Averaged daily urinary Na excretions were 320, 147 and 27 mEq after 5 days of high, normal and low Na regimens, respectively. Mean blood pressure increased by 7.9 mmHg following Na loading (p<0.05) and decreased by 5.6 mmHg following Na restriction (p<0.05) when compared with the value obtained under the normal Na regimen. Na restriction resulted in increases in PRA, Ald and PNE, while Na loading resulted in significant decreases in PRA and PNE. The change of Na intake had no significant effect on PE, TX \(B_2\) or 6-keto-PG \(F_{1\alpha}\). Body weight decreased slightly but significantly after Na restriction.

Figure 1 shows the changes of mean blood pressure and heart rate after the single oral administration of 40 mg nicardipine under the three different levels of Na intake. The mean blood pressure began to decrease significantly 10 min after nicardipine and reached its lowest level after between 60 to 80 min. The heart rate increased concomitantly with the decrease in blood pressure. The changes of mean blood pressure and heart rate after nicardipine were not different among the three Na intakes.

Changes of PRA, Ald, PNE, PE, TX \(B_2\) and 6-keto-PG \(F_{1\alpha}\) after nicar-
Table I. Clinical Findings in 15 Subjects with Essential Hypertension with High (27 g/day of salt), Normal (10 g/day of salt) and Low (0 g/day of salt) Na Regimens

<table>
<thead>
<tr>
<th>Na load</th>
<th>High</th>
<th>Normal</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>148±6*</td>
<td>138±5</td>
<td>122±4*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>89±2</td>
<td>84±5</td>
<td>77±3</td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>110±3*</td>
<td>102±1</td>
<td>96±3*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>63±3</td>
<td>65±3</td>
<td>63±3</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>60.7±2.6</td>
<td>60.6±2.6</td>
<td>59.6±2.6*</td>
</tr>
<tr>
<td>Plasma renin activity (ng/ml/h)</td>
<td>0.44±0.06*</td>
<td>0.58±0.15</td>
<td>2.90±0.83**</td>
</tr>
<tr>
<td>Serum aldosterone (pg/ml)</td>
<td>65.1±9.9</td>
<td>76.2±8.1</td>
<td>132.8±23.7**</td>
</tr>
<tr>
<td>Plasma norepinephrine (pg/ml)</td>
<td>146.9±15.9*</td>
<td>192.8±18.8</td>
<td>294.5±65.8*</td>
</tr>
<tr>
<td>Plasma epinephrine (pg/ml)</td>
<td>25.3±7.2</td>
<td>19.4±4.2</td>
<td>58.3±12.0</td>
</tr>
<tr>
<td>6-keto-prostaglandin F₁α (pg/ml)</td>
<td>43.1±8.3</td>
<td>88.6±29.5</td>
<td>75.3±25.1</td>
</tr>
<tr>
<td>Thromboxane B₂ (pg/ml)</td>
<td>77.8±29.8</td>
<td>60.4±12.8</td>
<td>58.5±12.0</td>
</tr>
<tr>
<td>Urinary Na excretion (mEq/day)</td>
<td>319.5±24.4**</td>
<td>146.8±7.0</td>
<td>26.6±6.2**</td>
</tr>
<tr>
<td>Urinary Cr excretion (mg/day)</td>
<td>1139±122</td>
<td>1184±114</td>
<td>1056±54</td>
</tr>
</tbody>
</table>

Cr=creatinine. Results are expressed as mean±SEM. * p<0.05, ** p<0.01 compared with values on normal Na regimen.

dipine are shown in Fig. 2. PRA was increased significantly by nicardipine under both normal and low Na regimens and tended to increase under the high Na regimen. Nicardipine brought about no change in Ald under either Na intake. PNE was increased by nicardipine under high and normal Na regimens, but PE was not changed by nicardipine under either Na intake. TX B₂ tended to increase after nicardipine, but the increase in TX B₂ was not statistically significant because of the wide variation of the measured values. There was no definite change in 6-keto-PG F₁α after nicardipine.

When the relationships between the change of mean blood pressure after nicardipine (ΔMBP) and measured variables were examined, ΔMBP correlated negatively with the pretreatment mean blood pressure (Fig. 3) under the three Na intakes. The slopes of the correlation line for high, normal and low Na regimens were −0.61, −0.69 and −0.52, respectively, without statistical significance. There was no definite relationship between ΔMBP and other variables, such as age and basal levels of PRA, Ald, PNE, PE, TX B₂ or 6-keto-PG F₁α.

**DISCUSSION**

In this study, changes of blood pressure and humoral factors after the single oral administration of nicardipine were determined in relation to Na intake in subjects with essential hypertension, for the purpose of clarifying the
Fig. 1. Changes of mean blood pressure and heart rate after a single oral administration of 40 mg nicardipine with different Na loads. \( \Delta \text{HR} = \) change of heart rate; \( \Delta \text{MBP} = \) change of mean blood pressure. Vertical bars denote 1 SEM. * \( p < 0.05 \), ** \( p < 0.01 \) compared with pretreatment values.

influence of Na balance on the hypotensive effect of Ca-antagonists. According to previous reports in which the factors responsible for the hypotensive effect of Ca-antagonists were investigated, the effects of Ca-antagonists were influenced by the pretreatment level of blood pressure, age of the subject, baseline value of PRA and change of Na balance. Erne et al.\(^7\) observed that the decrease of blood pressure after chronic treatment with nifedipine or verapamil correlated positively with pretreatment blood pressure and age, and correlated negatively with basal level of PRA. They propose that Ca-antagonists can be used as first-line antihypertensives, especially in older and low-renin patients. On the other hand, there are conflicting conclusions reported
by several investigators concerning the influence of Na intake on the hypotensive effect of Ca-antagonists. Valdés et al.\textsuperscript{8} investigated the depressor effect of a single oral administration of 20 mg nifedipine in subjects with essential hypertension who were under low (9 mEq/day of Na), normal (120 mEq/day of Na) and high (200 mEq/day of Na) Na diets. According to their data, the decreases of blood pressure after nifedipine under high and low Na diets were significantly smaller than that under a normal Na diet. The decreasing hypotensive effect of a Ca-antagonist during low Na intake can be explained by the greater vasopressor tone due to stimulation of the renin-angiotensin system, whereas during high Na intake it may be ascribed to increased arteriolar reactivity induced by Na loading. In another study by MacGregor et al.\textsuperscript{9} a single oral administration of 5 mg nifedipine induced a significantly larger depressor response in essential hypertensive patients who
were under high Na intake with a diet containing 350 mEq/day of Na, as compared with normal (150 mEq/day of Na) and low (10 mEq/day of Na) Na intakes. They proposed a possible mechanism by which high Na intake may trigger an increase in plasma levels of Na transport inhibitor and increase the concentrations of intracellular Na and Ca in arteriolar smooth muscle. In the present study, a significant correlation was found between changes of mean blood pressure after nicardipine and the pretreatment level of mean blood pressure under three levels of Na intake, indicating the magnitude of the untreated blood pressure may be a major determinant of the blood pressure fall due to Ca-antagonism. However, we cannot find any difference in the hypotensive effect of nicardipine under the three Na intakes. The levels of Na intake used in this study which were calculated from daily excretion of Na were equivalent to those of MacGregor et al\textsuperscript{9} under the high and normal Na regimens, but Na restriction in this study was less than that used by Valdés et al\textsuperscript{8} and MacGregor et al.\textsuperscript{9} The reasons why our results differ from previous reports with respect to the influence of Na intake on the depressor effect of Ca-antagonists, although not clear, may be related to differences in the selection of patients, drug used, dose of drug and Na balance.

The heart rate after nicardipine increased with a concomitant rise in PNE. The increase in heart rate by nicardipine may be due to an activation of the sympathetic nervous system in the face of peripheral vasodilation by a Ca-antagonist. However, PE was not changed after acute oral administration of nicardipine. This result agrees with the data reported by Defer
et al., in which an increase of PE after anaerobic exercise was attenuated during nifedipine therapy, despite the fact that an increase of PNE after exercise was significantly greater during nifedipine therapy compared to placebo treatment. Furthermore, it was reported that nifedipine resulted in a great improvement of cardiovascular symptoms with a pronounced decline in elevated urinary catecholamine in one patient with pheochromocytoma. These results as well as ours indicate that Ca-antagonists may directly inhibit catecholamine release from the adrenal medulla.

Another finding of this study is that Ald was not changed, even though a marked increase of PRA was observed under the three Na regimens after nicardipine administration. This result fits in with previous reports in which the changes of PRA and Ald were investigated during acute and chronic treatment with nifedipine and nitrendipine in subjects with essential hypertension. The mechanism by which Ca-antagonists stimulate renin release is speculated to be due to an indirect effect as a consequence of activated sympathetic outflow in the face of their peripheral vasodilatory properties and in part to direct action by the inhibition of Ca++ influx into juxtaglomerular cells. Ald secretion from the adrenal cortex induced by angiotensin II was Ca++ dependent and a Ca-antagonist inhibited Ald responses to angiotensin II and K+ in the isolated glomerulosa cells of rats. In a clinical study, pretreatment with nifedipine or diltiazem was reported to suppress the increase in the plasma level of Ald induced by intravenous infusion of angiotensin II. Both acute and chronic treatment with nifedipine resulted in well controlled blood pressure and normalized serum K+ with a concomitant fall of plasma Ald concentration in subjects with primary aldosteronism. These results as well as ours suggest that Ca-antagonists stimulate renin release from the kidney but inhibit Ald secretion from the adrenal gland. However, there was one report which showed that no definite change in PRA or Ald was observed during long-term antihypertensive therapy with Ca-antagonists.

There are several lines of evidence from in vitro experiments indicating that Ca++ has an important role in prostaglandin synthesis and metabolism in platelets and vascular walls. Ca-ionophore A23187 stimulates prostaglandin synthesis by the rat renal medulla and thromboxane production in washed human platelets. Ca-antagonists inhibit thromboxane synthesis in platelets and decrease angiotensin II-induced prostaglandin production in isolated dog renal arteries. However, there are few reports investigating the responses of prostaglandins during the clinical use of Ca-antagonists. Honda et al demonstrated that ratios of 6-keto-PGF1α to TX B2 increased significantly after intravenous administration of 2 mg nicardipine and suggested that
the shift in the ratio of vasoconstrictory thromboxane and vasodilatory prostacyclin metabolism might in part contribute to the hypotensive effect of Ca-antagonists. In another report of Uehara et al, plasma levels of TX B2 decreased after 2 to 8 weeks of treatment with 30 mg/day of nifedipine in patients with essential hypertension. In this study, we could not obtain any positive data supporting the concept that a change in prostaglandin metabolism may have a role in the hypotensive effect of Ca-antagonists, because nicardipine induces no definite change of plasma 6-keto-PG F1α or TX B2. A further detailed examination will be needed to clarify the possible role of prostaglandin metabolism in the hypotensive effect of Ca-antagonists.

REFERENCES
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