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# A cross-over medication trial for patients with autosomal-dominant hypertension with brachydactyly

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**A cross-over medication trial for patients with autosomal dominant hypertension with brachydactyly.** We examined a family with autosomal-dominant hypertension and brachydactyly from northeastern Turkey. The hypertension was defined as severe, resulting in stroke before age 50 years, featuring normal renin, aldosterone, and catecholamine responses, and did not appear to be salt-sensitive. The responsible gene resides on chromosome 12p. To determine which medications were most effective, we performed a prospective clinical trial. We studied 13 affected individuals in a randomized double-blind, cross-over trial including a beta-blocker (BBL), alpha-blocker (ABL), calcium channel blocker (CCB), converting enzyme inhibitor (CEI), and hydrochlorothiazide (HCT) and placebo (PLA). We then added moxonidine (MOX) and continued the trial for an additional period in a single-blind fashion. Each drug was given for four weeks with an option to double the dose after two weeks; each washout period comprised two weeks. Blood, 24-hour urine, and saliva were studied at the outset, and blood and urine samples were obtained at the end of each phase. Blood pressure (BP) and heart rate measurements were with the patient ambulatory at 24 hours. All regimens required doubled doses at two weeks. Beta blocker, CCB, CEI, and ABL lowered BP (6 to 10 mm Hg) and BP load compared to PLA, while HCT and MOX did not. Converting enzyme inhibitor and HCT increased plasma renin activity (PRA), while BBL lowered PRA. The 24-hour urine analysis indicated a high dietary salt intake with a low potassium and calcium intake. The salivary electrolytes showed similar sodium and potassium concentrations, while chloride values were significantly higher in affected than nonaffected subjects. Thus, this monogenic form of hypertension resembles nonsalt-sensitive essential hypertension in that BBL, CCB, CEI, and ABL were effective, while HCT was not. The BP reduction was similar to other single drug trials in essential hypertension. The high salivary chloride values suggest an additional intermediary phenotype that may be related to electrolyte transport. These results raise the possibility that an as yet unknown hypertensive mechanism is operative in these subjects.

Much has been learned about the pathogenesis of hypertension from unusual forms of monogenic hypertension such as glucocorticoid remediable aldosteronism [1], Liddle syndrome [2], and the syndrome of apparent mineralocorticoid excess [3]. In these diseases, the response to antihypertensive therapy was a major clue to which genes might be responsible for the individual syndromes [4]. We examined a large kindred from northeastern Turkey with a monogenic form of autosomal-dominant hypertension, which cosegregates with brachydactyly. Affected persons generally die of stroke before age 50 years [5]. We mapped the responsible gene(s) to chromosome 12p [6]. In an earlier clinical study, we found that the hypertension is elicited by a marked increase in peripheral vascular resistance, that the renin, aldosterone, and catecholamine values respond normally to volume expansion and contraction, and that affected individuals do not appear to be salt-sensitive [7]. With few exceptions, none of the affected family members were being treated for hypertension. We conducted a randomized, cross-over, placebo-controlled, double-blind trial of five medications, to determine the most effective pharmacological treatment. Since only a single gene, and thereby a single mechanism, is responsible for hypertension in this kindred, we reasoned that a single drug might be more effective in all subjects than all other drugs combined. A pharmacological phenotype might shed light on pathogenic mechanisms. We based our drug selection on medications recommended by the World Health Organization/International Society of Hypertension for the first-line treatment of hypertension, namely thiazide diuretics (HCT), beta blockers (BBL), alpha blockers (ABL), angiotensin converting enzyme inhibitors (CEI), and calcium channel blockers (CCB) [8]. Towards the end of the study, the imidazoline receptor agonist moxonidine (MOX) was made available to us, so we also tested that drug in the last phase of the study, albeit in a single-blinded fashion. The drugs were tested against a run-in phase, as well as against placebo (PLA). Twenty-four-hour ambulatory blood pressure measurements were made at the end of each phase, as well as 24-hour urine collections for electrolyte excretion and blood samples for serum chemistries. To our knowledge, this trial is the first randomized double-blind, placebo-controlled cross-over study of "first line" antihypertensive medications.

**Key words:** genetics, hypertension, monogenic hypertension, anti-hypertensive therapy, salivary electrolytes.

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## METHODS

We recruited 17 family members with hypertension and brachydactyly. The protocol was approved of the internal review boards of both the Hacettepe University, Ankara, Turkey, and the Humboldt University of Berlin, Germany. Informed written consent in the Turkish language was obtained from all participants.

The inclusion criteria were affected status (brachydactyly), blood pressure > 140/90 mm Hg for subjects ≥ 16 years and > 95 percentile for age (> 136/> 86 mm Hg) for subjects < 16 years of age [9]. Three subjects were < 16 years old: a 13-year-old girl, a 15-year-old boy and a 15-year-old girl. Exclusion criteria were secondary hypertensive complications (stroke, heart disease, renal disease), pregnancy, or plans to become pregnant and lactation. We elected to study several younger individuals if they were hypertensive and were willing and able to participate. We did so knowing the prognosis of the disease and also because we had decided to treat all subjects > 35 years routinely to prevent stroke. The 5 men and 12 women, none of whom had ever received antihypertensive treatment, ranged in age from 13 to 34 years. A Turkish physician and Turkish speaking medical student moved to the home village of the subjects and lived there for 12 months during the course of the study. They performed all the examinations, obtained the blood and urine samples, and were available to the subjects for any problems, whether or not they were related to the study.

A pill count was conducted every two weeks of the study. Compliance was defined as no more than a two tablet error in terms of pill count. Furthermore, at that time the subjects were questioned extensively regarding their general health, their energy levels and the presence of fatigue, any changes in their psychological well being, their general level of function, and satisfaction with their physical abilities. The answers were monitored and comparisons of the various phases were made at the end of the study.

After the initial subject selection, the subjects were observed for one month without active medication, but with placebo tablets identical to those described below. Then they underwent 24-hour ambulatory blood pressure and heart rate monitoring (Space Labs 90217-2; Space Labs Inc., Redmond, WA, USA). Measurements were taken every 15 minutes during the day and half-hourly at night. We accepted 24 hour measurements with less than 20% missing values, although for the majority of subjects we encountered less than 10% missing values. Day was defined as 0600 to 2200 hours, while night was defined as 2200 to 0600 hours. This time interval corresponded well with the subjects' work and sleeping habits. The subjects were also instructed in the collection of a 24-hour urine specimen for sodium, potassium, calcium, and creatinine excretion analysis. On the following morning, the subjects were weighed and measured and their blood was obtained for electrolytes, plasma renin activity (PRA), and plasma aldosterone (ALD) values. Electrolytes and creatinine in blood and urine were measured by automated techniques. Plasma renin activity and ALD were measured by radioimmunoassay. In addition to the above examinations, we also collected saliva from the 17 subjects, as well as from 10 nonaffected relatives. The saliva was gathered between meals in the morning; some subjects chewed on rubber bands to assist in stimulating saliva. Sodium, potassium and chloride were measured in the saliva in order to determine any additional phenotypic characteristics of the subjects that might have to do with electrolyte transport [10].

Thereafter, the subjects were randomized to one of six regimens (PLA, HCT, BBL, ABL, CEI, or CCB) for a two week period, at the end of which they were examined by the physicians. If their blood pressures were above the study entry criteria, the subjects were moved to a higher dose of that regimen. At the end of another two weeks, the 24-hour ambulatory blood pressure and heart rate measurements, the urine collections, and the blood specimens were repeated. A two-week washout period was begun, at the end of which another of the six regimens was initiated. During the washout period, the subjects were rotated to PLA medication. Thus, they ingested the same appearing capsule and tablet after the run-in period for the entire duration of the study. At two-weekly intervals, the subjects were questioned in detail about possible side effects. Towards the end of the study, we decided to include a trial of MOX therapy. Since that regimen could no longer be randomized into the study, we gave MOX after the last regimen in the same fashion and in the same capsule and tablet combination, as all the other regimens. All subjects reported in this study participated in all seven regimens, including MOX. We gave the following medications and doses: hydrochlorothiazide 25 mg/day, metoprolol (zero order kinetic single daily dose formulation) 50 or 100 mg/day, doxasosin 2 or 4 mg/day, ramipril 2.5 or 5 mg/day, felodipine 5 or 10 mg/day, and moxonidine 0.2 or 0.4 mg/day.

To more precisely estimate the stress which the cardiovascular tree experienced during each blood pressure-measurement day, we calculated the blood pressure load. The blood pressure load encompasses not only the blood pressure level above a certain threshold, but also the time of exposure. The threshold has been established as > 140/90 mm Hg for adults. The blood pressure load has been shown to correlate with complications better than casual blood pressure determinations [11]. Blood pressure load was calculated as the mean area under the blood pressure curve (AUC) for all values above a 140/90 mm Hg threshold for subjects > 16 years. For subjects < 16 years of age, we calculated the area under the blood pressure curve for all values > 136/86 mm Hg. The blood pressure load value is expressed as mm Hg [11]. Twenty-four hour AUC was divided by time to form blood pressure load as a measure of total vessel overload. We did not distinguish the load in terms of diurnal and nocturnal values, since we were interested in stress on the vasculature rather than in the circadian variation *per se*. We also indicated the percent of all blood pressure determinations during 24 hours, which were above the threshold values.

Statistical analysis was conduced using SPSS (SPSS Inc., Chicago, IL, USA). Effects of medications were tested using repeated measures analysis of variance. Different medications were tested against run-in and placebo by pair-wise *t*-tests with Bonferoni's correction. To examine differences in response to the various medications, the medication effect order was tested by Friedman's rank test. The test allows the gerenal determination of differences in effect, although differences between specific treatments cannot be discerned. The data are presented as mean ± SD. A $P$ value $<0.05$ was considered statistically significant.

## RESULTS

Twelve subjects completed the trial. One 28-year-old woman developed marked dizziness and light headedness during the first phase of the treatment and requested that the therapy be stopped. She agreed to continue in the study for the next and subsequent phases and did so successfully, although we did not include her data in the analysis. When we unblinded her treatment, we learned that she had ingested PLA during her episodes of light headedness. Three other young women left the study after they announced plans to marry and had altered their pregnancy plans. Another man was not able to participate in the MOX segment of

**Table 1.** Blood pressure (mm Hg) and heart rate (beats/min) results obtained from 24-hour ambulatory measurements in the subjects (mean ± SD)

| Variable | RUN | BBL | CCB | CEI | ABL | HCT | MOX | PLA | *P* |
|---|---|---|---|---|---|---|---|---|---|
| SYS 24 hr | 148 ± 18 | 135 ± 18[ab] | 137 ± 13[ab] | 138 ± 15[ab] | 140 ± 16[a] | 142 ± 18[a] | 144 ± 15 | 145 ± 16 | 0.01 |
| DIA 24 hr | 97 ± 13 | 87 ± 13[ab] | 90 ± 11[ab] | 91 ± 12[ab] | 91 ± 12[a] | 94 ± 15 | 94 ± 13 | 95 ± 13 | 0.01 |
| HR 24 hr | 74 ± 5 | 65 ± 6[ab] | 77 ± 7 | 75 ± 7 | 77 ± 8 | 76 ± 4 | 73 ± 5 | 75 ± 5 | 0.01 |
| SYS day | 153 ± 18 | 129 ± 19[ab] | 141 ± 14[ab] | 142 ± 15[ab] | 143 ± 16[a] | 146 ± 19[a] | 147 ± 14 | 148 ± 16 | 0.01 |
| DIA day | 101 ± 13 | 90 ± 14[ab] | 93 ± 11[a] | 93 ± 11[ab] | 94 ± 12[a] | 97 ± 15 | 97 ± 12 | 98 ± 13 | 0.01 |
| HR day | 77 ± 6 | 67 ± 7[ab] | 81 ± 8 | 78 ± 8 | 81 ± 9 | 79 ± 6 | 76 ± 6 | 78 ± 5 | 0.01 |
| SYS night | 132 ± 19 | 119 ± 17[ab] | 120 ± 14[ab] | 124 ± 17[a] | 125 ± 18 | 123 ± 15[a] | 132 ± 20 | 128 ± 17 | 0.01 |
| DIA night | 84 ± 14 | 74 ± 13[a] | 77 ± 12[a] | 80 ± 14 | 80 ± 15 | 79 ± 14[a] | 85 ± 15 | 82 ± 15 | 0.01 |
| HR night | 65 ± 6 | 59 ± 5[ab] | 63 ± 6 | 63 ± 7 | 65 ± 6 | 64 ± 4 | 64 ± 5 | 65 ± 5 | 0.05 |
| SYS load mm Hg | 13 ± 12 | 6 ± 9[ab] | 6 ± 6[ab] | 7 ± 8[ab] | 8 ± 8[a] | 9 ± 10 | 10 ± 11 | 10 ± 10 | 0.01 |
| DIA load mm Hg | 11 ± 8 | 5 ± 6[ab] | 5 ± 5[a] | 6 ± 6[a] | 7 ± 6[a] | 9 ± 9 | 9 ± 8 | 9 ± 8 | 0.01 |
| SYS load % | 64 ± 30 | 40 ± 31[ab] | 45 ± 27[ab] | 47 ± 30 | 50 ± 33 | 51 ± 33 | 56 ± 25 | 60 ± 29 | 0.01 |
| DIA load % | 70 ± 25 | 46 ± 31[ab] | 54 ± 25[a] | 58 ± 26[a] | 58 ± 30 | 63 ± 28 | 63 ± 27 | 65 ± 26 | 0.01 |
| SYS day/night | 21 ± 5 | 19 ± 12 | 21 ± 8 | 18 ± 7 | 19 ± 12 | 23 ± 7 | 15 ± 9 | 20 ± 10 | NS |
| DIA day/night | 17 ± 6 | 16 ± 7 | 16 ± 7 | 13 ± 7 | 14 ± 9 | 18 ± 6 | 12 ± 9 | 16 ± 8 | NS |
| SYS rank | — | 2.9 | 3.3 | 3.4 | 3.7 | 4.3 | 5.1 | 5.4 | 0.05 |
| DIA rank | — | 2.3 | 3.3 | 3.7 | 3.6 | 4.8 | 4.8 | 5.4 | 0.05 |
| HR rank | — | 1.1 | 4.7 | 3.9 | 4.8 | 5.1 | 3.8 | 4.6 | 0.05 |

Abbreviations are: RUN, run in; BBL, beta blocker; CCB, calcium channel blocker; CEI, converting enzyme inhibitor; ABL, alpha blocker; HCT, hydrochlorothiazide; MOX, moxonidine; PLA, placebo. Blood pressure load is expressed as the area under the curve of all values above a given threshold as mm Hg and the % of all values above the threshold as described in Methods.

[a] Significantly different from RUN

[b] Significantly different PLA

the study. His data were also not included in the analysis. The pill counts showed that the subjects' compliance was excellent. All regimens met our criteria for compliance. Generally, there were no errors. The standardized questionnaires to establish possible side effects were evaluated and no differences among the regimens could be identified. We excluded all data on subjects failing to complete all arms of the study so that we could employ a repeated measures analysis of variance approach. However, by so doing, we reduced our numbers of subjects accordingly. The subjects ranged in age from 13 to 34 years. Five were men and eight were women. The body mass index ranged from 20.3 to 26.8.

Table 1 shows the 24-hour blood pressure and heart rate data for the run-in phase and seven treatments. The dose of the drugs required doubling at two weeks in every instance. Thus, the higher dose was tested at four weeks. Systolic and diastolic blood pressure, heart rate, daytime and night time blood pressures and heart rates, as well as the systolic and diastolic blood pressure loads all showed a significant effect of treatment. The day/night blood pressure difference (dipping phenomenon) was not influenced by treatment. All subjects exhibited an appropriate diurnal blood pressure variation. We were able to assign rank order scores to the treatments compared to PLA. For systolic blood pressure these were: BBL, CCB, CEI, ABL, HCT, MOX, and PLA. The rank order for diastolic blood pressure was little different. The BBL treatment had a significant effect on heart rate.

Figure 1 shows the daytime values from 24-hour blood pressure and heart rate measurements in the same rank order as given in Table 1. Significant differences between drug regimens and run-in, as well as significant differences with placebo, are indicated. Beta blockers, CCB, CEI, and ABL lowered blood pressure compared to PLA, while HCT and MOX did not. Figure 2 shows the 24-hour blood pressure load. The blood pressure load was significantly reduced for the same regimens, compared to PLA. We also sought to find effects of age and could identify none.

Table 2 shows the electrolyte values in blood and urine, as well as PRA and ALD determinations. Plasma electrolytes, including calcium and phosphate, were not influenced by any of the regimens. Plasma renin activity and ALD increased significantly with HCT treatment compared to the other regimens; PRA increased significantly with CEI treatment, while ALD did not. Renin values with the BBL treatment were significantly lower than with the PLA treatment. The 24-hour urine collections showed considerable variability, probably indicating compliance difficulties. The 24-hour sodium and potassium excretion rates were constant under the regimens as would be predicted by steady state. The 24-hour calcium excretion was variable, and although the values decreased numerically with HCT compared to run-in, significance could not be shown. The salivary sodium values in nonaffected persons were 10.4 ± 3.2 compared to 14.5 ± 9.4 mmol/liter in affected subjects; the corresponding potassium values were 21.4 ± 5.5 versus 24.3 ± 5.6 mmol/liter, respectively. On the other hand, the corresponding chloride values were 16.9 ± 5.0 versus 24.8 ± 7.6 mmol/liter, respectively ($P < 0.05$). A comparison between anions and cations (anion gap) between the two groups ($[Na^+K]$-Cl) revealed values of 14.9 ± 4.8 versus 14.1 ± 6.3 mmol/liter, respectively, which were not significantly different.

## DISCUSSION

The important findings in this study were that BBL, CCB, CEI and ABL lowered blood pressure in these subjects with monogenic hypertension and brachydactyly, while HCT and MOX did not. These findings are consistent with the suggestion that this form of monogenic hypertension resembles salt-resistent essential hypertension. To our knowledge, this is the first double-blind, cross-over study involving the agents suggested as first-line drugs for the treatment of essential hypertension. Clearly, it is the first such study employing 24-hour blood pressure monitoring. Although our study was relatively brief, the blood pressure reductions we observed with BBL, CCB, CEI, and ABL were similar in magnitude than those reported in the Treatment of Mild Hypertension (TOMHS) Study [12]. That investigation had a parallel

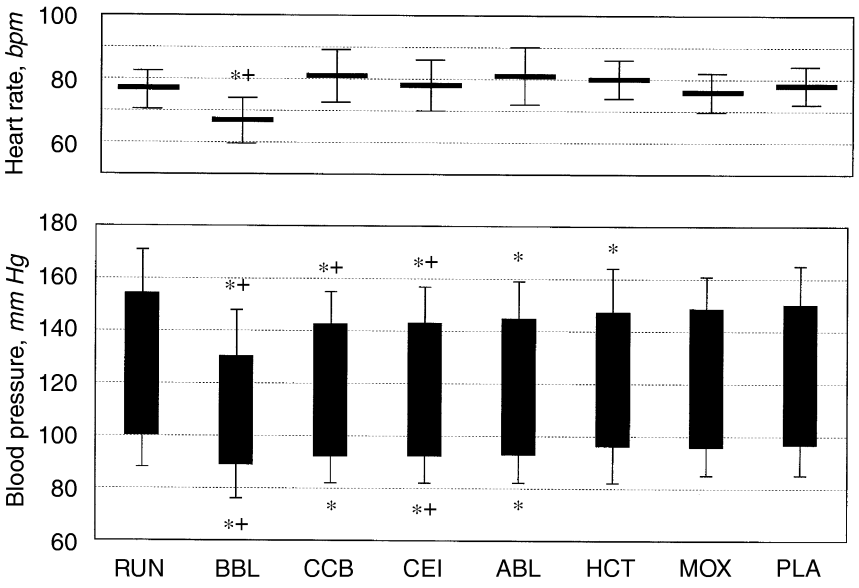


**Fig. 1. Day-time values from 24-hour ambulatory blood pressure measurements and heart rate in the subjects at the end of each regimen.** Significant differences between treatment and run-in (*$P < 5\%$ vs. RUN), and treatment and placebo (+$P < 5\%$ vs. PLA), are indicated.

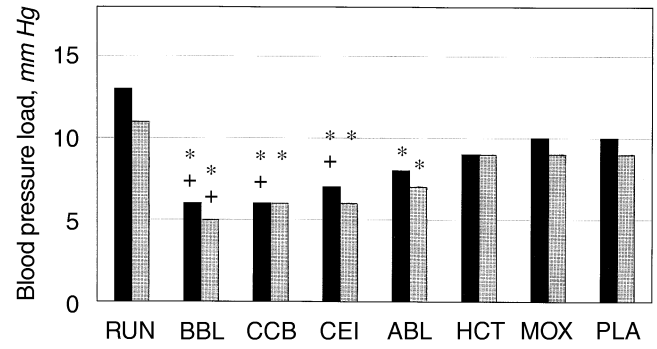


**Fig. 2. Twenty-four hour blood pressure load determined at the end of each regimen**. Symbols are: (■) systolic BP load; (▨) diastolic BP load. Significant differences between treatment and run-in (*$P < 5\%$ vs. RUN), and treatment and placebo (+$P < 5\%$ vs. PLA), are indicated.

design, the numbers of subjects were far greater, and the period of observation extended to four years.

We had hoped to find a single treatment that would give us insight into the mechanism of this hypertensive syndrome. In glucocorticoid remediable aldosteronism, the response to low dose of prednisone, response to thiazide diuretics, response to spironolactone, and the appearance of abnormal steroid urinary metabolites gave important clues as to what genetic mechanisms were operative [13]. Liddle himself suggested, on the basis of his patients' response to antihypertensive treatment, what the nature Liddle syndrome was likely to be [14]. The resemblance of apparent mineralocorticoid excess to licorice gluttony, as well as the response to thiazide diuretics and to spironolactone elucidated the candidate gene that proved to be responsible [15]. On the other hand, our results do not point to an obvious candidate gene. However, we do not view our results as a "negative" study any more than we would consider the TOMHS results to be a negative study [11]. The fact that we found no straightforward pharmacological phenotype suggests that this monogenic hypertension may be of particular relevance to essential hypertension.

The cross-over design is powerful; however, the small number of subjects in our study and the considerable variability in blood pressure are limiting factors. The hypertension characterized by these subjects greatly age-dependent. The fact that we included three subjects $< 16$ years of age increased the blood pressure variability. These persons were the least hypertensive and thereby exhibited the mildest blood pressure reductions with treatment. We did not feel justified in including persons $> 35$ years of age in our study, because of the well documented risk of stroke in this family. We treated such persons with two or three drug regimens, generally with a BBL, CCB, CEI combination.

We conducted pill counts that suggested that our subjects were compliant. Also facilitating compliance was our close relationship with the family and their very clear awareness of the consequences of their disease. Furthermore, we observed a decrease in PRA with BBL therapy, an increase in PRA with CEI therapy, and an increase in both PRA and ALD with HCT therapy. These expected reactions support the notion that the medications were being ingested and were effective.

Hydrochlorothiazide failed to significantly reduce blood pressure in our subjects. One interpretation of this result is that persons with autosomal-dominant hypertension with brachydactyly are indeed not salt-sensitive. Our earlier phenotypization study used an accepted protocol to determine salt-sensitivity and resistance and give similar results [7]. An alternative view is that the substantial salt intake of our subjects made the chances for HCT therapy remote. The urinary sodium excretion of our subjects ranged from 177 to 256 mmol/day. These amounts exceed even the highest salt intakes observed in the 52 centers of the Intersalt study [16]. However, the dose of HCT we administered (25 mg/day) is the dose currently recommended and has been used effectively in major intervention trials [17]. The fact that the renin-agniotensin system was influenced by thiazide treatment attests to volume effects produced by that drug.

The 24-hour urine collections of our subjects revealed not only

**Table 2.** Serum chemistries, plasma renin activity, plasma aldosterone, and urine chemistries in the subjects at the end of each phase (mean ± SD)

| | RUN | BBL | CCB | CEI | ABL | HCT | MOX | PLA | *P* |
|---|---|---|---|---|---|---|---|---|---|
| Sodium *mmol/liter* | 138 ± 4 | 140 ± 4 | 139 ± 4 | 141 ± 4 | 140 ± 6 | 139 ± 4 | 140 ± 1 | 141 ± 3 | NS |
| Potassium *mmol/liter* | 4.3 ± 0.6 | 4.5 ± 0.4 | 4.5 ± 0.6 | 4.4 ± 0.5 | 4.4 ± 0.6 | 4.2 ± 0.7 | 4.3 ± 0.4 | 4.6 ± 0.5 | NS |
| Chloride *mmol/liter* | 105 ± 3 | 105 ± 2 | 104 ± 3 | 104 ± 2 | 105 ± 2 | 104 ± 2 | 102 ± 2 | 106 ± 3 | NS |
| Calcium *mmol/liter* | 2.4 ± 0.2 | 2.3 ± 0.2 | 2.4 ± 0.1 | 2.4 ± 0.2 | 2.4 ± 0.1 | 2.3 ± 0.1 | 2.3 ± 0.1 | 2.3 ± 0.1 | NS |
| Phosphate *mmol/liter* | 1.3 ± 0.7 | 1.2 ± 0.2 | 1.2 ± 0.2 | 1.2 ± 0.2 | 1.1 ± 0.2 | 1.2 ± 0.2 | 1.1 ± 0.2 | 1.1 ± 0.2 | NS |
| Creatinine *mg/dl* | 0.94 ± 0.30 | 0.88 ± 0.15 | 0.93 ± 0.18 | 0.91 ± 0.16 | 0.86 ± 0.10 | 0.94 ± 0.19 | 0.86 ± 0.17 | 0.91 ± 0.14 | NS |
| Aldosterone *ng/ml* | — | 0.35 ± 0.18 | 0.54 ± 0.39 | 0.38 ± 0.17 | 0.35 ± 0.22 | 0.88 ± 0.67 | 0.50 ± 0.23 | 0.46 ± 0.29 | 0.05 |
| Renin *ng/Ang I/hr* | — | 1.2 ± 0.8[a] | 2.7 ± 2.2 | 7.1 ± 5.4[a] | 1.8 ± 0.9 | 4.5 ± 3.3 | 1.8 ± 1.1[a] | 2.7 ± 1.3 | 0.05 |
| 24 hr Creatinine *g/24 hr* | 0.91 ± 0.29 | 0.75 ± 0.27 | 0.81 ± 0.33 | 0.77 ± 0.32 | 0.82 ± 0.37 | 0.67 ± 0.25 | 0.76 ± 0.31 | 0.75 ± 0.35 | NS |
| 24 hr Potassium *mmol/24 hr* | 36 ± 21 | 40 ± 22 | 28 ± 14 | 37 ± 14 | 41 ± 25 | 28 ± 14 | 31 ± 8 | 39 ± 18 | NS |
| 24 hr Sodium *mmol/24 hr* | 177 ± 59 | 239 ± 107 | 199 ± 68 | 244 ± 131 | 286 ± 114 | 244 ± 127 | 244 ± 68 | 256 ± 134 | NS |
| 24 hr Calcium *mg/24 hr* | 99 ± 52 | 84 ± 72 | 115 ± 82 | 93 ± 63 | 91 ± 45 | 57 ± 45 | 70 ± 49 | 86 ± 53 | NS |

Abbreviations are in the legend to Table 1.
[a] Significantly different from PLA

a very generous salt intake, but also a surprisingly modest intake of potassium and calcium. Both have been suggested to be important to blood pressure regulation [18, 19]. Potassium intake has been particularly related to the occurrence of stroke [20]. An avenue for nonpharmacological intervention in terms of modifying electrolyte intake may be possible in affected family members.

It was interesting to find an elevated salivary chloride in our subjects compared to nonaffected family members. We do not believe this finding to be artifactual, since the sodium and potassium values were not different and since the electrolyte relationships (salivary anion gap) were maintained. Skrabal et al [10] found earlier that young salt-sensitive persons had lower salivary sodium concentrations compared to salt-resistant individuals. These findings prompted the present measurements. We have no explanation for this interesting intermediary phenotype as yet. The 6 cm segment on chromosome 12p currently receiving our attention [21] does contain potential candidate genes for channels. Genes related to the L-type calcium channel lie outside of our segment. The gene for the adenosine 5′-triphosphate (ATP)-dependent potassium channel lies within our segment; however, we sequenced the three exons of that gene and found no mutations. The ATP-dependent potassium channels have important effects on vascular tone [22]. Nevertheless, the smooth muscle cell sufonyl urea receptor gene, which regulates the ATP-dependent potassium channel, is very close in proximity and remains a candidate [23]. Interestingly, the sulfonyl urea drug glibenclamide exercises inhibitory effects on the cystic fibrosis transmembrane regulator and can thereby influence chloride channels [24]. We plan additional patch clamp studies on fibroblasts and lymphoblasts from our subjects to further elucidate this possibility.

We were surprised that MOX was not more effective in lowering blood pressure in our subjects. We had included MOX because of our observation that affected individuals exhibit an anomalous looping posterior inferior cerebellar artery, which could be impinging on the ventrolateral medulla. We conducted magnetic resonance imaging investigations in 15 affected and 11 nonaffected members of this family and found such anomalies in all affected members, while no nonaffected persons had such findings [25]. These anomalies, termed neurovascular compression, may elicit a neurogenic hypertension [26]. Neurovascular compression may be readily identified with magnetic resonance imaging and has been found to be more prevalent in patients with essential hypertension than in those with secondary hypertension [27]. The fact that MOX was not effective in these subjects by no means rules out a possible central nervous system cause for the hypertension; however, supportive evidence for this hypothesis from MOX treatment or ABL therapy was not forthcoming in our study. We have no specific reason to believe that the MOX formulation we employed was deficient in terms of bioavailability. However, since we did not monitor drug serum concentrations, we cannot be certain that effective concentrations were achieved.

On the basis of our findings, we have begun treating our subjects permanently. Most of our subjects are receiving a BBL plus CEI or BBL plus CCB combination therapy. Women desiring to become pregnant have been advised to take the BBL treatment. We hope to be able to prevent stroke in our subjects with antihypertensive therapy. Since we have reasonable actuarial information on this family from prior generations, it is quite conceivable that we will be able to assess the effect of our treatment in the future. All affected family members are now receiving antihypertensive therapy.

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## APPENDIX

Abbreviations used in this article are: HCT, thiazide diuretics; BBL, beta blockers; ABL, alpha blockers; CEI, angiotensin converting enzyme inhibitors; CCB, calcium channel blockers; MOX, moxonidine; PLA, placebo; AUC, area under the blood pressure curve; BP, blood pressure; PRA, plasma renin activity; ALD, plasma aldosterone.

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