Effects of Dietary γ-Linolenic Acid on Blood Pressure and Adrenal Angiotensin Receptors in Hypertensive Rats (44292)

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Abstract. **In a previous study, we showed that dietary gamma-linolenic acid (GLA), an omega-6 polyunsaturated fatty acid found in borage oil (BOR), attenuates the development of hypertension in young spontaneously hypertensive rats (SHR). The purpose of this study was to determine the effects of dietary GLA on established hypertension in adult rats, as well as its effects on components of the renin-angiotensin-aldosterone axis. For 5 weeks, male SHR (14–15 weeks old) were fed a basal fat-free diet to which 11% by weight of sesame oil (SES) or BOR was added. Systolic blood pressure (SBP), determined by the tail cuff method, and weight were measured weekly. Plasma renin activity (PRA), aldosterone (PA), and corticosterone (PC) levels were measured at the end of the dietary treatments. The adrenal glands were homogenized, and angiotensin II (ANG II) binding was measured and plotted according to Scatchard. Systolic blood pressure was 12 mmHg lower at Week 5 in SHR fed the BOR diet compared to SES-fed rats ($P < 0.005$). Weight gains were similar in both dietary groups. Plasma aldosterone was lower, PRA was higher, and the PA/PRA ratio was significantly lower ($P < 0.05$) in BOR-fed rats. Levels of PC were the same in both groups. The BOR-enriched diet reduced adrenal ANG II receptor density and affinity compared to the SES diet. Results suggest that BOR inhibits adrenal responsiveness to ANG II by an action on adrenal receptors. Our findings demonstrated that dietary GLA lowers SBP in adult SHR. This effect may be mediated, at least in part, by interference with the renin-angiotensin-aldosterone system at the level of adrenal ANG II receptors.** [P.S.E.B.M. 1998, Vol 218]

Hypertension is a major cause of death and cardiovascular disease in westernized societies (1). Nutritional measures may lower blood pressure with fewer side effects than traditional drugs. Among these measures are diets rich in the omega-6 polyunsaturated fatty acid gamma-linolenic acid (GLA) (2, 3). Previous studies have demonstrated that evening primrose oil, which contains 8%–14% GLA, has an antihypertensive effect on rodents, including spontaneously hypertensive rats (SHR) (4–7) and stressed and salt-loaded rats with borderline hypertension (8, 9). Borage oil, which contains 18%–25% GLA, has been shown to lower blood pressure in normotensive rats (10). Moreover, borage oil attenuates the development of hypertension in young SHR (7, 11). The present study was undertaken to evaluate the effects of dietary borage oil in SHR with established hypertension. We also investigated the effects of dietary borage oil on the renin-angiotensin-aldosterone axis *in vivo* and *in vitro*.

Materials and Methods

Animals and Diet. Male SHR (14–15 weeks old) were obtained from Harlan (Indianapolis, IN) and housed

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This work was supported in part by the Department of Veterans Affairs and National Institutes of Health Grant #HL 11046.

Received November 5, 1997. [P.S.E.B.M. 1998, Vol 218]
Accepted February 25, 1998.

0037-9727/98/2183-0234$10.50/0

two to three per cage at constant temperature (26°C), humidity (60%), and lighting (12-hr dark/light cycle). The rats were randomly assigned to two groups and fed a diet for 5 weeks of a fat-free basal mix (Teklad, Madison, WI) with the addition of 11% by weight of either sesame oil (SES) (Amend Drug & Chemical Co., Irvington, NJ) or borage oil (BOR) (Traco Labs, Seymour, IL). Dietary ingredients and the fatty acid composition of the oils are presented in Tables I and II. The oils were used to provide similar proportions of total 18-carbon monounsaturated and polyunsaturated fatty acids in the diet yet control for GLA, 18:3n6. The content of linoleic acid (18:2n6) was 44% ± 5%. The control SES-based diet was rich in oleic acid (18:1n9) at 35% and devoid of GLA. The BOR-based diet contained oleic acid (14%) and GLA (24%). The fatty acid compositions was determined as previously described (12), using a capillary gas chromatograph (Hewlett-Packard Model 5890A) and a 15-mm × 0.5-mm fused silica capillary column (Supelco, Inc., Bellefonte, PA) with hydrogen as the carrier gas at a linear velocity of 167 cm/sec. The diets were prepared weekly and stored at 0°C. Fresh provisions of the diet were administered daily. Body weights were measured weekly. The study was conducted in accordance with the guidelines of the Committee on Animal Research at the University of California-San Francisco.

Blood Pressure Measurement. Systolic blood pressure was determined by a photoelectric tail cuff system (Model 179, IITC, Inc., Woodland Hills, CA) at room temperature (10). Blood pressure was measured prior to the dietary treatments and weekly thereafter. The values presented were the average of three separate readings.

Measurement of Plasma Aldosterone, Corticosterone, and Renin Activity. Following 5 weeks of dietary treatment, the rats (aged 19–20 weeks) were anesthetized with a mixture of oxygen (70%), nitrous oxide (30%), and halothane (5%). Blood samples were collected after cardiac puncture into prechilled tubes containing EDTA. Plasma was separated by centrifugation at 800 rpm for 20 min at 1°C. The plasma samples were stored at −70°C until assayed. Plasma renin activity was measured as the rate of angiotensin I generation in plasma incubated for 3 hr at pH 6.5 (13). Plasma aldosterone and corticosterone levels were determined by radioimmunoassay (14, 15).

Table I. Dietary Ingredients

Ingredients	Diet (g/kg)
Casein	225
DL-methionine	3
Sucrose	492
Corn starch	169
Cellulose	56
Vitamin mix[a]	11
Mineral mix[b]	39
Calcium carbonate	5
Fat[c]	110

[a] Teklad AIN-76.
[b] Teklad 40060.
[c] Fat added as sesame oil or borage oil.

Table II. Composition of Major Fatty Acids in the Oils[a]

Fatty acid	% Total fatty acids	
	Sesame oil	Borage oil
Palmitic	9.3	9.0
Stearic	5.5	3.3
Oleic	35.1	14.3
Linoleic	48.1	39.5
Gamma-linolenic	[b]	24.4
Alpha-linolenic	0.4	0.1

[a] Values are expressed as percent of total fatty acids.
[b] Not detected.

Measurement of Adrenal Angiotensin II Receptor Binding. Adrenal glands were removed, and the capsules were isolated, frozen on dry ice, and stored immediately at −70°C. To prepare thawed membranes, tissue samples were transferred into chilled Kreb's buffer solution (containing in m*M*): NaCl, 136.9; KCl, 3.6; $MgCl_2$, 5.0; dextrose, 11.1; HEPES, 20.0; EGTA, 13.4; PMSF, 2.83; 8-hydroxyquinoline, 3.44; and bacitracin, 10 IU/ml (pH 7.4). This mixture was homogenized using a Brinkman Polytron (Model PT10-20—3500). The adrenal capsules were homogenized twice with 5-sec pulses separated by chilling in an ice bath (1 min). The suspension was centrifuged at 1000*g* for 10 min, the supernatant was decanted and saved on ice, and the pellet was resuspended in Kreb's buffer solution and homogenized by three pulses. This suspension was sedimented as described above. The resulting supernatant was pooled with the previous sample and used in the binding assays.

Aliquots of pooled supernatant were pipetted into tubes with graded amounts of unlabeled Sar^1 angiotensin II or ligands for receptor subtypes, and the mixture was vortexed briefly. The subtype-specific ligands were losartan for AT_1 and PD 123319 for AT_2, both added to a final concentration of 5 μg/ml. Following incubation for 5 min at 22°C, ^{125}I-Sar^1 angiotensin II was added and mixed, and the suspension was incubated for 30 min at 22°C. Membranes and bound angiotensin II were pelleted by centrifugation at 27,000*g* for 30 min. The supernatant was aspirated, and the tube sides were washed with 3 ml of ice-cold buffer. After the wash, the tube's contents with the pellet were counted in a gamma well scintillation counter.

After counting, pellets were dried overnight at 110°C and rehydrated with NaOH (0.1 *N*, 100 μl). The tubes were capped tightly and incubated at 37°C for 72 hr. The resulting solutions were pooled, mixed, and quantitated for protein using a Coumassie blue protein reagent kit (Pierce Chemical Co., Rockford, IL).

Statistical Analysis. Results are expressed as mean ± SEM. Data were evaluated for statistical significance with the Crunch statistical package (Crunch Software, Oakland,

CA), using Student's t test for unpaired data. Analysis of variance with repeated measures was also used. Significant effects were evaluated further by Scheffe's *post hoc* tests at a significance level of 0.05.

Results

Body Weight and Blood Pressure. Body weights did not differ significantly between groups at the end of the 5-week dietary treatments {381 ± 3 g (SES) and 374 ± 6 g (BOR)}. Systolic blood pressure was significantly lower in BOR-fed SHR at Weeks 3, 4, and 5 compared to the SES-fed SHR (Fig. 1). The BOR-enriched diet reduced mean blood pressure at Week 5 by 12 mmHg ($P < 0.005$).

Plasma Aldosterone, Corticosterone, and Renin Activity. Plasma aldosterone was lower, and plasma renin activity was higher in BOR-fed rats compared to SES-fed rats, but the differences were not statistically significant (Table III). As a consequence, the ratio of plasma aldosterone to renin was significantly lower in the BOR-fed SHR ($P < 0.05$). Corticosterone levels in the two groups did not differ significantly.

Adrenal Angiotensin II Binding. The effects of dietary BOR on binding of ligands to angiotensin receptors from SHR adrenal glomerulosa cells are shown in Figure 2. The BOR-enriched diet resulted in lower angiotensin II receptor density compared to the SES diet. Binding to SES membranes was inhibited 88% by the AT_2-specific ligand losartan ($P < .001$), and 31% by the AT_2-specific ligand PD 123319 ($P < .01$). Binding to BOR membranes was inhibited 36% by the AT_2 ligand ($P < .01$) and 94% by the AT_1 ligand ($P < .001$). These results showed that angiotensin receptors in adrenal capsules were predominantly of the AT_1 subtype in both groups of rats.

Discussion

Results of this study show that borage oil reduced the blood pressure of adult SHR by 12 mmHg; this reduction was observed as early as 3 weeks following the start of borage oil enrichment. These results extend our previous observations that dietary borage oil treatment prevented the onset of hypertension in prehypertensive, 6–7-week-old SHR (7, 11). Thus, dietary borage oil is an effective antihypertensive nutrient in the developmental and established stages of hypertension in SHR.

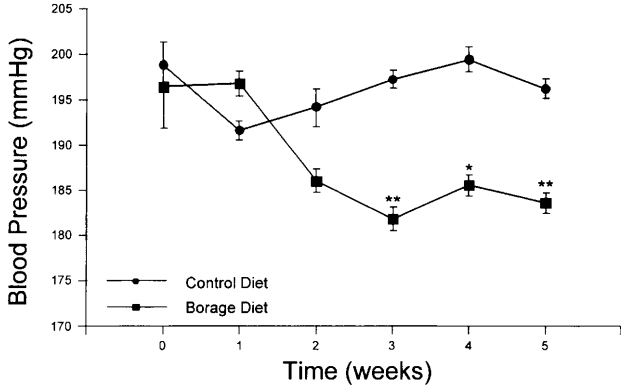

Figure 1. Effect of dietary borage oil on systolic blood pressure in SHR. * = $P < 0.01$, ** = $P < 0.005$, vs SHR which were fed sesame oil–enriched control diet. Each point represents the mean ± SEM of five rats.

Table III. Plasma Aldosterone, Corticosterone, Renin Activity and Ratio in SHR Rats Fed Sesame Oil– or Borage Oil–Enriched Diets[a]

Parameter	Dietary group	
	Sesame oil	Borage oil
Plasma corticosterone (mg/dl)	40 ± 1	46 ± 8
Plasma aldosterone (ng/dl)	10.0 ± 1.4	7.9 ± 0.8
Plasma renin activity (ng/ml/hr)	6.9 ± 0.6	9.6 ± 1.8
Ratio of aldosterone/renin activity	1.45 ± 0.2	0.88 ± 0.1[b]

[a] Values are expressed as mean ± SEM, $n = 5$ for each group.
[b] $P < 0.05$, vs SES-fed SHR.

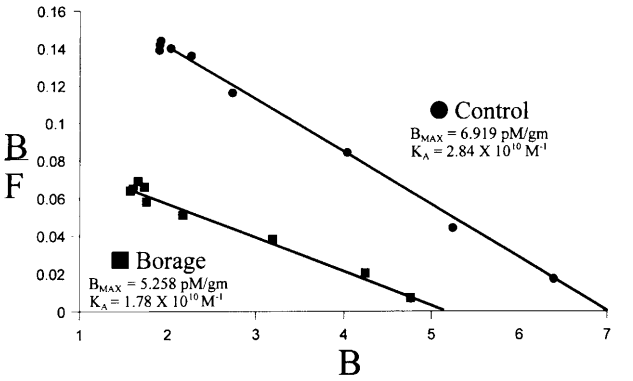

Figure 2. Scatchard plot of adrenal angiotensin II receptor binding in SHR rats fed sesame oil– or borage oil–enriched diets. "B" refers to bound ligand, and "F" refers to unbound (free) ligand at equilibrium.

To investigate the role of the renin-angiotensin-aldosterone axis in the blood pressure response, we determined levels of plasma aldosterone and renin activity. Plasma aldosterone was lower, and plasma renin activity was higher in BOR-fed SHR compared to SES-fed SHR, resulting in a significant reduction in the plasma aldosterone/renin ratio. This finding suggests that borage oil reduces the responsiveness of rat adrenal glomerulosa cells to angiotensin. Renin secretion is stimulated by the decreased aldosterone levels, and the net effect is reduced aldosterone levels despite the rise in renin.

To investigate responsiveness to angiotensin more directly, we quantitated angiotensin receptors in adrenal glands from the two groups of rats. The number and affinity of antagonist receptors in adrenals were lower in rats fed borage oil compared to sesame oil. These findings suggest that the reduced plasma aldosterone/renin ratio in rats fed borage oil is the result of diminished angiotensin receptor activity in the cells that produce aldosterone. Previous studies have shown that unesterified fatty acids inhibit adrenal and vascular smooth muscle angiotensin II receptors (16, 17). Dietary borage oil may exert its effects on angiotensin

receptors by increasing levels of free GLA, or by modifying the fatty acid composition and physicochemical properties of cell membranes. Omega-6 polyunsaturated fatty acids increase membrane fluidity, which in turn influences membrane receptor function (18). We recently reported that dietary treatment with borage oil for 7 weeks increases the levels of omega-6 polyunsaturated fatty acids, gamma-linolenic, and dihomo-gamma-linolenic acids in plasma, liver, aorta, and renal arterial tissue of SHR (19, 20). The precise mechanism by which dietary GLA reduces angiotensin receptor function cannot be elucidated from our data and requires further investigation.

An effect on aldosterone-producing cells might explain part or all of the pressure-lowering property of dietary borage oil. Competitive inhibitors of aldosterone action, such as spironolactone, lower the blood pressure of adult SHR (21). Arterial pressure could also be lowered by inhibition of extra-adrenal angiotensin receptors. The receptors inhibited by BOR were primarily of the AT_1 subtype; this subtype mediates angiotensin's stimulation of vasoconstriction, sympathetic transmission, and renal tubular sodium absorption, as well as production of aldosterone (22). If dietary borage oil affected any of those extra-adrenal responses, it would have contributed to the pressure-lowering effect we observed.

In conclusion, our findings demonstrate that dietary borage oil, rich in GLA, lowers blood pressure in SHR with established hypertension. The blood pressure response is associated with a reduction in the affinity and density of angiotensin II receptors in cells that produce aldosterone, and a reduction in the plasma aldosterone/renin ratio. The results suggest that the blood pressure effect of borage oil may be mediated by interference with the renin-angiotensin-aldosterone system.

We are grateful to DuPont/Merck for the losartan, and to Parke-Davis and Company for the PD 123319 used in these experiments. We thank Mr. Sid Tracy of Traco Labs and Mr. G. J. de Wit of Zaadteelt, Zaadhandel, Texel, the Netherlands, for the borage oil.

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