Cardiovascular and Sympathetic Responses to Chronic Arachidonate in SHR and WKY Rats

MOHAMED A. BAYORH, PH.D., ZOFIA ZUKOWSKA-GROJEC, M.D., DAVID EZRA, M.D., GIORA Z. FEUERSTEIN, M.D., AND IRWIN J. KOPIN, M.D.

SUMMARY In rats between the ages of 4 and 12 or 14 weeks, repeated daily subcutaneous administration of arachidonate (AA) at a dose of 50 or 200 mg/kg significantly retarded the development of hypertension in spontaneously hypertensive rats (SHR) but did not alter the normal age-related increase in blood pressures (BP) of normotensive (WKY) rats. Heart rates (HR) and plasma levels of norepinephrine (NE), but not epinephrine, were lower in AA-treated SHR than in saline-treated animals. AA-treated SHR and WKY gained less weight than the saline-treated controls. In pithed AA-treated SHR, stimulation of the sympathetic outflow (50 V, for 1 minute at 0.3 or 3.0 Hz) and intravenous administration of NE (0.3 or 3.0 g/kg) evoked smaller pressor responses than in saline-treated controls, but the stimulation-evoked increases in plasma catecholamines were unchanged by AA treatment. These results indicated that, in SHR, chronic AA treatment reduces BP by mechanisms that do not directly affect NE release from sympathetic nerves. There appears to be both reduced central nervous system activation of the sympathetic outflow and diminished responses to peripheral sympathetic stimulation and exogeneous NE which may be secondary to the reduced vascular hypertrophy that usually accompanies the development of high BP in SHR.

(Hypertension 5: 172-179, 1983)

KEY WORDS • arachidonic acid • conscious SHR • blood pressure • heart rate • plasma catecholamines • hypertension

PROSTAGLANDINS appear to be involved in the complex mechanisms that regulate the cardiovascular system. They act directly on blood vessels, the heart, sympathetic nervous system, and on release of renin-angiotensin and vasopressin, as well as mediating the effects of the pressor agents. Abnormal prostaglandin metabolism has been implicated in the pathogenesis of the elevated blood pressure (BP) in spontaneously hypertensive rats (SHR). Thus, arterial tissue from SHR releases more PGE_2 and PGI_2 than does vascular tissue from normotensive (WKY) rats. Furthermore, the activity of the rate-limiting enzyme of arachidonate (AA) metabolism, phospholipase A_2, as well as several prostaglandin-degrading enzymes vary markedly between SHR and WKY rats.

We have recently shown that the hypotensive response to administered AA is greater in conscious SHR than in WKY rats. Since this response is abolished by indomethacin, we concluded that the enhanced hypotensive effect of AA in SHR is mediated by cyclooxygenase products of AA. These findings raised the question of whether or not the enhanced sensitivity of SHR to AA can be sustained over prolonged periods. This possibility is suggested by several studies in which high BP development in SHR and stroke-prone SHR is attenuated by feeding a modified fat diet. Although the specific dietary factor responsible for the beneficial effects of modified fat diets is still obscure, excess of specific polyunsaturated fatty acids could well contribute to the observed hypotensive effect. Furthermore, recent studies showing that certain human populations that consume diets rich in polyunsaturated fatty acids have a reduced incidence of cardiovascular disease.

The following study was undertaken to examine the effects of chronic administration of AA on the ontogenesis of high BP in SHR, the effect of AA supplementation on the peripheral vascular reactivity to pressor agents, and the activity of the sympathetic nervous system as assessed by assay of plasma levels of norepinephrine (NE) and epinephrine in conscious rats and in pithed SHR during stimulation of sympathetic impulse outflow from the spinal cord.
Material and Methods

Experimental Procedures

Male SHR and WKY rats (3 weeks old, weighing 50 to 60 g) were obtained from Taconic Farms (Germantown, New York). Animals were housed five per cage with food and water ad libitum for 1 week in a room with 12-hour light-dark cycles. After a week of acclimatization, a preliminary experiment was conducted in which each group of SHR and WKY rats was subdivided into two equal subgroups. One subgroup of SHR and WKY rats treated with AA, (50 mg/kg, s.c. in saline, given Monday, Wednesday, and Friday, each week for 13 weeks), whereas, the other group received a comparable volume of saline. In the preliminary study, BP was measured 36 hours after the last dose of AA.

In a second set of experiments, 10 SHR 4 weeks old received a daily injection of AA (200 mg/kg, s.c.) 7 days a week, and another group of 10 SHR received an equal volume of saline. BPs were measured with tail-cuff plethysmography using a Grass polygraph model 5 (Grass Instrument Company, Quincy, Massachusetts) and a programmed Electro-sphygmomanometer PE-300 (Narco, Bio-System, Inc., Houston, Texas). Heart rates (HR) were determined before treatment (at 4 weeks of age) and every other week for 8 weeks (until 12 weeks of age).

At the age of 14 weeks, drug or saline administration was stopped and indwelling, tail-artery catheters (PE-50) were inserted in all rats under halothane anesthesia (2% oxygen) as previously described.16 Twenty-four hours were allowed for recovery from the surgical procedure before direct BP and HR were recorded (Beckman 4-327-C pressure transducer, Dynograph 511 A, Beckman Instruments, Inc, Fullerton, California). The BP and HR were recorded continuously for 30 minutes while the rats were conscious and unrestrained in their cages (which were surrounded with a screen to minimize stress from external stimuli). For the measurement of plasma catecholamines in awake animals, blood samples (0.4 ml) were withdrawn from each rat into heparinized glass tubes. Blood samples were treated as described below for pithed rats.

In a third experiment, 28 12-week-old SHR were divided into four groups: control (AA vehicle); arachidonic acid (AA); control + indomethacin and AA + indomethacin; and treated with either AA 200 mg/kg, s.c. and/or indomethacin 4 mg/kg, s.c. two times daily. Control groups received equal volumes of bicarbonate or bicarbonate + indomethacin for 21 days. Direct BP levels in conscious rats were determined as previously described.

Stimulation of Sympathetic Outflow from Spinal Cord of Pithed Rats

The animals were anesthetized with 2% halothane in oxygen; the right jugular vein was cannulated (PE-50 polyethylene tubing) for drug administration, and both vagi severed at the cervical level. Both carotid arteries were cannulated with polyethylene tubing (PE-50): one for recording BP and HR and the other for collection of blood samples. Following cannulation of the trachea, the rats were pithed by insertion of a steel rod (1.5 mm diam) through the orbit and foramen magnum, down the spinal cord to the first sacral vertebra for a total distance of 15 cm, and arterial respiration immediately instituted. This procedure destroys the entire central nervous system but leaves intact the emerging nerve trunks. Gallamine (Flaxedil, 20 mg/kg, i.v.) was injected 15 minutes after pithing to prevent muscle contractions during stimulation. Another steel rod was inserted under the skin of the back from the left shoulder to the right hind limb to serve as an indifferent electrode. Fifteen minutes after the administration of gallamine, the entire sympathetic nervous system was stimulated for 1 minute using monophasic square wave pulses (50 V, 1 msec duration) at 0.3 Hz, and, again, 15 to 20 minutes later at 3.0 Hz. Blood samples (0.4 ml) were collected from each animal prior to and during the last 15 seconds of each spinal cord stimulation. Fifteen minutes after the last stimulation, doses of norepinephrine (NE), 0.3 and 3.0 /ig/kg, were administered intravenously with a 15-minute interval between doses. At the end of the experiment animals were sacrificed and the hearts, livers, kidneys, spleens, and both adrenals, were removed and weighed separately.

Collection and Storage of Blood Samples in Pithed Rats

In experiments requiring multiple blood samples, blood volume was maintained by replacement of blood obtained from other rats. Blood samples were collected by free flow via the polyethylene cannula in the left carotid artery into heparinized glass tubes and centrifuged at 5000 × g for 10 minutes at 4°C. Aliquots of plasma (150 pl) were mixed with 150 f11M of 0.5 M perchloric acid containing 31.8 mM EGTA and centrifuged as above. Aliquots (200 f11A) of the protein-free supernatant were stored at —20°C until assayed.

Assay of Norepinephrine and Epinephrine in Plasma

Plasma content of NE and epinephrine was assayed by a radioenzymatic thin-layer chromatographic procedure as previously reported.1819 In brief, protein-free aliquots of plasma were incubated with catechol-0-methyl transferase and tritiated S-adenosyl methionine. After incubation, the reaction was stopped by the addition of borate buffer (pH 8) containing metanephrine and normetanephrine. The amines were extracted into toluene-isoamyl alcohol (3:2) and then into 0.1 M acetic acid. The radioactive products were separated by thin-layer chromatography, the appropriate areas identified under ultraviolet light, and the areas separately scraped into counting vials. After peridate oxidation of the O-methylated compounds to vanillin, phosphor-containing toluene was added and the tritium content determined by liquid scintillation spectrometry.
Drugs

The following drugs were used in this study: arachidonic acid (5, 8, 11, 14-Eicosatetraenoic acid, 99%) and 1-norepinephrine bitartrate (Sigma Chemical Company, St. Louis, Missouri). Both drugs were dissolved in saline.

Statistical Evaluation

Multivariant analysis of variance for repeated measures was used for statistical evaluation of systolic BP, HR, and body weight of saline- and AA-treated SHR from the second part of the study. In the third study, one-way analysis of variance and the Student Newman-Keuls test were performed. The Student t test was applied for the analysis of other hemodynamic and biochemical data, as specifically noted in legends. Results are mean values ± SEM.

Results

Effects of Chronic Arachidonate Treatment on Blood Pressure and Heart Rate of SHR

As shown in figure 1A, systolic BP of the 4-week-old SHR (n = 29) was significantly higher than in the WKY (n = 30) rats (95 ± 2 and 88 ± 2 mm Hg respectively, p < 0.01). Twelve weeks after the AA treatment (50 mg/kg s.c. three times weekly), SHR had significantly lower systolic BP (tail-cuff plethysmography) than saline-injected SHR: 187 ± 4 vs 203 ± 6 mm Hg respectively (fig. 1A, p < 0.02). There were no significant differences in the BP of AA- and saline-treated WKY rats (fig. 1A).

Prior to treatment, the HR of the 4-week old SHR was significantly higher than that of WKY rats (491 ± 10 vs, 450 ± 9 bpm respectively, p < 0.01). In both the SHR and WKY rats, HR decreased with age and no effect of arachidonate was observed (fig. 1B).

In the second study, the mean pretreatment systolic BP of 4-week-old SHR was: 109 ± 3 mm Hg (fig. 2A). Saline-treated SHR developed a more marked hypotension than did SHR treated with 200 mg/kg/day s.c. of AA for 8 weeks.

From 8 to 13 weeks of age, the systolic BP (tail-cuff plethysmography) of AA-treated SHR was significantly lower than that of the saline-injected SHR (178 ± 4 and 206 ± 4 mm Hg respectively, at the end of this period). The trend of the changes of systolic BP was significantly different in the saline- and AA-treated SHR (F = 7.12, p < 0.002). At 14 weeks, 1 day after termination of treatment, directly measured systolic BP of conscious, unrestrained, AA-treated SHR was even lower than in saline-injected SHR; 149 ± 4 vs 199 ± 7 mm Hg respectively, (p < 0.001, fig. 2A).

In both groups of SHR, the HR declined similarly throughout 4 to 13 weeks of age (fig. 2 B, F = 24.5, p < 0.001, and F = 207.3, p < 0.001, AA- and saline-injected SHR respectively). However, during direct measurement through the tail-artery cannula in conscious, unrestrained rats, a significantly lower HR was found in the AA-treated than in the saline-injected SHR (304 ± 11 and 374 ± 16 bpm respectively, p < 0.002, fig. 2B).

Effect of Subchronic Arachidonate and Indomethacin Treatments on Blood Pressure of Conscious SHR

In the third experiment, the systolic BP of AA-treated SHR was significantly lower than that observed after saline injections (178 ± 5.8 vs 211.0 ± 7.3 mm Hg respectively, p < 0.01). Indomethacin alone did not significantly alter BP, but in combination with AA it almost completely reversed the hypotension induced by AA (178.7 ± 5.8 for AA vs 202.0 ± 3.7 mm Hg for AA + indomethacin, p < 0.05, table 1).

Effect of Chronic Arachidonate Treatment on Body Weight of SHR and WKY Rats

In both SHR and WKY rats, the injections of AA (50 mg/kg s.c. three times weekly) resulted in signifi-
ARACHIDONATE IN HYPERTENSION/Bayorh et al. 175

**TABLE 1. Effect of Chronic Arachidonate (AA) and Indomethacin Treatments on the Blood Pressure of Conscious SHR**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Systolic BP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>211.0±7.3</td>
</tr>
<tr>
<td>AA</td>
<td>178.7±5.8*</td>
</tr>
<tr>
<td>Control + indomethacin</td>
<td>216.4±7.9</td>
</tr>
<tr>
<td>AA + indomethacin</td>
<td>202.0±3.7†</td>
</tr>
</tbody>
</table>

Statistical analysis was by one-way analysis of variance: F = 7.298, p < 0.001; and the Student-Newman-Keuls test was used for multiple comparison among groups. The level of statistical significance of differences is denoted by *p < 0.01 control vs AA-treated, †p < 0.05 AA vs AA + indomethacin.

**Figure 2. Effect of chronic arachidonate (AA) treatment (200 mg/kg) on systolic blood pressure (A) and heart rate (B).** Multivariate analysis of variance for repeated measures revealed significant differences in trends. A. For group and period effect between saline- and AA-treated SHR, F = 7.12, p < 0.002. B. For period effect within the groups of saline- or AA-treated SHR, F = 24.5, p < 0.001 and F = 207.3, p < 0.001 respectively. Directly measured systolic blood pressure and heart rate of 14-week-old AA-treated SHR were significantly lower than those of saline-treated SHR (p < 0.001, panel A, and p < 0.02, panel B respectively).

**Figure 3. Effect of chronic arachidonate treatment on body weight of SHR and WKY rats.** Multivariate analysis of variance for repeated measurements showed significant differences in trends for group and period effect between saline- and AA-treated SHR, F = 10.27, p < 0.001. Student t test revealed a significant difference in body weights of 12-week-old saline vs AA-treated WKY rats (p < 0.01).

Effect of Chronic Arachidonate Treatment on Plasma NE and EPI in Conscious SHR

At the end of the interval of AA treatment, basal plasma catecholamines were measured in the arterial blood of conscious, unrestrained SHR while they were resting in their cages. AA-treated SHR had significantly lower plasma NE than saline-injected SHR (62.8 ± 8.7 and 111.9 ± 15.2 pg/ml respectively, p < 0.01), whereas no difference in plasma epinephrine levels were found between those two groups of rats: (21.0 ± 5.6 and 17.5 ± 3.0 pg/ml respectively).

Effect of Chronic Arachidonate Treatment on Pressor Responses of Pithed SHR

After pithing, the mean BP of AA-treated SHR was not significantly lower than that of saline-injected animals (58 ± 2 and 65 ± 2 mm Hg respectively, fig. 4). Low (0.3 Hz) frequency of stimulation of the spinal cord induced significantly smaller pressor responses in significantly lower body weights than in the respective saline-injected controls (fig. 3, p < 0.001 and p < 0.01 for SHR and WKY respectively). At the end of the experimental period, the weights of AA-treated SHR were similar to those of saline-treated WKY rats (233 ± 3 g and 231 ± 8 g respectively).

The treatment of SHR with the higher dose of AA (200 mg/kg/day) produced a similar degree of retardation of body weight increase as did the lower dose. At 13 weeks of age, AA-treated SHR weighed 222 ± 7 g as compared to 266 ± 7 g weight of saline-injected SHR (p < 0.001). The weight of AA-treated SHR in the second study did not differ from the weight of the AA-treated SHR in the first study at the end of the 12-week treatment period.

The weights of heart, kidney, liver, spleen, and adrenals of AA-treated SHR were proportionately smaller than those of saline-injected SHR so that the ratios of organ/body weight remained the same in both groups (table 2).
TABLE 2. Body and Organ Weights of Arachidonate (AA)- and Saline-Treated SHR

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Heart (× 10^2 mg)</th>
<th>Kidney (× 10^2 mg)</th>
<th>Liver (× 10^2 mg)</th>
<th>Spleen (× 10^2 mg)</th>
<th>Adrenal (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>225.9 ± 6.5</td>
<td>915.8 ± 33.6</td>
<td>1029.6 ± 38.5</td>
<td>1140.7 ± 54.9</td>
<td>858.1 ± 41.7</td>
<td>32.5 ± 0.01</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>± 33.6</td>
<td>± 38.5</td>
<td>± 54.9</td>
<td>± 41.7</td>
<td>± 0.02</td>
<td>± 0.002</td>
</tr>
<tr>
<td>saline</td>
<td>276.7 ± 8.2</td>
<td>1062.1 ± 22.7</td>
<td>1255.9 ± 99.7</td>
<td>1348.1 ± 570</td>
<td>880.4 ± 49.3</td>
<td>38.5 ± 0.13</td>
</tr>
<tr>
<td>(n = 9)</td>
<td>± 22.7</td>
<td>± 99.7</td>
<td>± 570</td>
<td>± 49.3</td>
<td>± 0.01</td>
<td>± 3.4 ± 0.02</td>
</tr>
</tbody>
</table>

Levels of statistical significance between AA- and saline-treated SHR (Student t test): *p < 0.02; **p < 0.001.

AA-treated SHR than in saline-injected SHR (the increments of mean BP being 17 ± 2 vs 35 ± 3 mm Hg respectively (p < 0.001). The pressor response to high (3.0 Hz) frequency stimulation was also significantly attenuated (+ 77 ± 6 in AA-treated SHR and + 106 ± 7 mm Hg in saline-injected rats, p < 0.05).

The pressor responses to administered NE (fig. 4) in doses of 0.3 and 3.0 μg/kg i.v. were also significantly lower in AA-treated than in saline-injected SHR. In AA-treated SHR, 0.3 μg/kg of NE evoked an increase in BP of 25 ± 4 mm Hg while in saline-injected SHR the increase was 40 ± 4 mm Hg (p < 0.05). The higher dose of NE (3.0 μg/kg) induced an increase in mean BP of 92 ± 5 and 118 ± 5 mm Hg in AA- and saline-treated SHR respectively (p < 0.01, fig. 4).

Discussion

The present study demonstrates that chronic administration of AA attenuates the development of high BP in SHR. These findings substantiate the enhanced hypotensive response of SHR to acute administration of AA.10 The hypotensive effect of chronic AA administration in SHR resembles previous findings showing that high-fat diets are effective treatment in this experimental model of hypertension.11-13 High-fat high-cholesterol diets were found to prevent the development of severe hypertension in stroke-prone SHR, with significant reduction in the incidence of neurological deficits and strokes.11-13 Also, Wexler14 has shown that SHR fed a high-fat diet at an early age failed to develop arterial hypertension up to 180 days of age, and that the high-fat diet induced marked endocrine and metabolic derangements including involution of the pituitary-gonadal axis.
The hypotensive effect of AA obtained in SHR is effective in attenuating the increase of BP in the spinal cord evoked similar release of NE and epinephrine in edem in this report indicate that both central and peripheral sites are involved. The lower levels of circulating NE in the conscious AA-treated SHR suggests that the AA-treated SHR also had a lower cardiac output. This assumption, however, awaits confirmation by direct measurement.

The mechanism of the reduced HR in AA-treated SHR may be related to excess production of prostaglandins of the E or F series. These prostaglandins stimulate vagal afferent receptors in the cardiopulmonary region and elicit vasodepression accompanied by bradycardia. The lack of difference in HR of pithed AA- or saline-treated SHR (in which all reflexes are eliminated) suggests a reflex origin (and perhaps central involvement) for the lower HR in the AA-treated rats.

The reduced body weight that attended chronic AA administration could also affect the ontogenesis of the hypertension. However, the relationships between body weight per se and the systemic BP are complex. Weight loss induced by caloric restriction was shown to reduce the high BP of SHR. Reduction of body
weight of obese human hypertensives facilitates the reduction of high BP and enhances the hypertensive treatment.\textsuperscript{30} In the present study, the relative contribution of weight loss to the hypertensive effect of AA cannot be determined precisely. AA-treated WKY rats also showed a significant retardation of growth (the relative reduction of body weight was 13%) but did not have lower systemic BP as compared to saline-treated WKY rats. AA-treated SHR showed a significant reduction of BP, yet the relative reduction in body weight (17.2%) was comparable to that of AA-treated WKY rats. It is also pertinent to note that SHR in this study have accelerated growth in body weight which is consistent with previous findings.\textsuperscript{33} Thus, it is obvious that excessive gain in body weight is not necessary for development of hypertension in SHR. This notion is in agreement with Wexler's study showing excess body weight in WKY vs SHR; yet the latter group developed hypertension.\textsuperscript{34}

The mechanism involved in the AA-induced growth retardation is not clear. Prostaglandins of the E series suppress food intake\textsuperscript{30} probably through interference with hypothalamic regulation of food intake.\textsuperscript{35} However, other mechanisms, e.g., reset of metabolic rate or enhanced renal clearance of salt and water induced by specific prostaglandins\textsuperscript{36, 37} may also contribute to the reduced body weight.

In conclusion, the present study demonstrates that pharmacological doses of AA given over prolonged periods of time retard the increase in BP in developing SHR. Several factors appear to contribute to the overall hypertensive effect of AA, including reduction of sympathetic outflow, reduced peripheral vascular structural changes that enhance responsiveness to pressor agents, lesser cardiac hyperactivity, and reduction of body weight. This, however, does not exclude the role of other cardiovascular control mechanisms not tested in this study.

References


25. Feuerstein G, Kopin U: Effect of PGD\textsubscript{2}, PGE\textsubscript{2} and PGF\textsubscript{2} on blood pressure, heart rate and plasma catecholamine responses to spinal cord stimulation in pithed rats. Prostaglandins 21: 189, 1981


Cardiovascular and sympathetic responses to chronic arachidonate in SHR and WKY rats.
M A Bayorh, Z Zukowska-Grojec, D Ezra, G Z Feuerstein and I J Kopin

Hypertension. 1983;5:172-179
doi: 10.1161/01.HYP.5.2.172
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/5/2/172

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/