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

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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.

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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,1 the heart,2 sympathetic nervous system,3 and on release of renin-angiotensin4 and vasopressin,5 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 PGE2 and PGH2 than does vascular tissue from normotensive (WKY) rats. Furthermore, the activity of the rate-limiting enzyme of arachidonate (AA) metabolism, phospholipase A2,6 as well as several prostaglandin-degrading enzymes7 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.8 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.9-14 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.15 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.²⁶ Twenty-four hours were allowed for recovery from the surgical procedure before direct BP and HR were recorded. 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 /g/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 X g for 10 minutes at 4°C. Aliquots of plasma (150 µl) were mixed with 150 fA of 0.5 M perchloric acid containing 31.8 mM EGTA and centrifuged as above. Aliquots (200 µl) 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.²² 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-isooamyl 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 0-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 1 A, 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. 1 A, p < 0.02). There were no significant differences in the BP of AA- and saline-treated WKY rats (fig. 1 A).

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. 1 B).

In the second study, the mean pretreatment systolic BP of 4 week-old SHR was: 109 ± 3 mm Hg (fig. 2 A). 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. 2 A).

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. 2 B).

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-
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...
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 weight (x 10^2 mg)</th>
<th>Kidney weight (x 10^2 mg)</th>
<th>Liver weight (x 10^2 mg)</th>
<th>Spleen weight (x 10^2 mg)</th>
<th>Adrenal weight (x 10^2 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>225.97 ± 6.5</td>
<td>915.8 ± 33.6</td>
<td>1029.6 ± 38.5</td>
<td>11407 ± 5.04</td>
<td>858.1 ± 0.38</td>
<td>32.5 ± 0.9</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>276.7 ± 8.2</td>
<td>1062.1 ± 0.38</td>
<td>1255.9 ± 0.45</td>
<td>13481 ± 0.46</td>
<td>880.4 ± 0.31</td>
<td>38.5 ± 0.01</td>
</tr>
<tr>
<td>Saline</td>
<td>225.97</td>
<td>915.8 ± 33.6</td>
<td>1029.6 ± 38.5</td>
<td>11407 ± 5.04</td>
<td>858.1 ± 0.38</td>
<td>32.5 ± 0.9</td>
</tr>
<tr>
<td>(n = 9)</td>
<td>276.7</td>
<td>1062.1 ± 0.38</td>
<td>1255.9 ± 0.45</td>
<td>13481 ± 0.46</td>
<td>880.4 ± 0.31</td>
<td>38.5 ± 0.01</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 ± 2 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).

Basal mean HR of AA- and saline-injected pithed SHR was similar (219 ± 9 and 229 ± 9 bpm respectively). There were no differences between AA- and saline-treated SHR in the response of HR to low and high frequency of stimulation (+104 ± 7 vs +111 ± 7 bpm respectively, at 3.0 Hz). The tachycardic response to administered NE was also similar in AA- and saline-treated SHR at both doses tested (+75 ± 7 and +88 ± 6 bpm, after the higher dose of NE).

Plasma Levels of NE and EPI in Pithed SHR After Chronic Arachidonate Treatment

Basal levels of plasma NE were similar in AA- and saline-injected pithed SHR (50 ± 8 and 38 ± 5 pg/ml, fig. 5). Furthermore, both groups of rats responded to low- and high-frequency stimulation with comparable increases in plasma NE. At 0.3 Hz the increments were 50 ± 17 and 46 ± 10 pg/ml in AA- and saline-treated SHR respectively, while at 3.0 Hz they were 976 ± 11 and 1086 ± 93 pg/ml respectively.

There was no significant differences in the stimulation-induced increases of plasma epinephrine between AA- and saline-injected SHR. At 0.3 Hz increases were 55 ± 10 vs 47 ± 5 pg/ml, and at 3.0 Hz they were 1963 ± 142 vs 2040 ± 182 pg/ml respectively (fig. 5).

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. 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. 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. Also, Wexler 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, fatty liver, and elevated circulating levels of lipids and enzymes. The attempt to replace a high-fat high-cholesterol diet by a specific polyunsaturated fatty acid, AA, proved that this fatty acid alone is effective in attenuating the increase of BP in the SHR. The hypotensive effect of AA obtained in SHR may be specific to this type of experimental hypertension, since administration of AA to renal hypertensive rats exacerbated the hypertension, and cyclooxygenase inhibitors prevented this effect.

The mechanism involved in the AA-induced reduction of the systemic BP is not clear. Specific metabolites of AA seem to be involved since a linolenic acid rich diet has no effect on the systemic BP of SHR. Although the mechanism by which chronic AA reduced the high BP in SHR is not clear, the data presented 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 activity of the sympathetic nervous system is reduced. Since hyperactivity of the sympathetic nervous system is considered to be an early and important pathophysiological mechanism for the elevated BP in SHR, reduced sympathetic activity may contribute to the attenuation of the hypertension. The reduced sympathetic tone is not the result of impaired peripheral sympathetic nerves (pre- or postganglionic) activity since stimulation of sympathetic outflow from the spinal cord evoked similar release of NE and epinephrine in pithed AA- or saline-treated SHR. Thus, a central role for the reduced sympathetic tone in AA-treated rats is suggested. Although various prostaglandins injected into the cerebroventricular system or specific brain nuclei stimulate the sympathetic nervous outflow and increase the circulating levels of both NE and epinephrine, the effect of endogenously found prostaglandins (PG) are unknown. Inhibition of NE release from sympathetic nerve endings by the metabolites of AA (PGE_2 series) could also explain the lower circulating levels of NE in AA-treated SHR. However, AA treatment would be expected to reduce release of NE in the spinal cord stimulated pithed rats. The data obtained in this study indicate that release of NE is not reduced. Since plasma NE responses to sympathetic stimulation was not different in pithed rats, it is highly unlikely that altered disposition (volume of distribution, plasma clearance, etc.) is responsible for the 50% reduction in plasma NE levels of awake AA-treated rats. Other recent studies also failed to demonstrate any presynaptic inhibitory effect of PGE_2 or PGI_2 on catecholamine release.

The major mechanism involved in the attenuation of the increase in BP in AA-treated SHR may be due to reduced responsiveness of the vascular bed to pressor stimuli. This reduction in responsivity may be due to prevention of structural changes in resistance vessels, either as a direct consequence of pressure reduction or indirect effect of diet. We observed a substantial reduction of the pressor response to spinal cord stimulation, which is mediated mainly by \( \alpha_1 \) adrenoceptors. Sympathetic stimulation has been previously shown to result in greater BP responses in pithed SHR than in normotensive WKY rats. Chronic AA treatment also reduced the BP response to administered NE, which involves both \( \alpha_1 \) and \( \alpha_2 \) adrenoceptors.

These results are similar to recent reports demonstrating that stroke-prone SHR fed a high-fat, high-cholesterol diet have lower BP and reduced pressor responses to intravenous administration of noradrenaline.

Additional mechanisms which may contribute to the hypotensive effect of AA include reduced cardiac output and weight loss. SHR were previously shown to have faster HR and greater cardiac outputs than normotensive WKY or Wistar rats. As early as 28 days of age, SHR in the present study had higher heart rates than WKY rats. The significant reduction of HR in AA-treated SHR (measured through the tail artery line) in addition to the lower systolic and diastolic BP 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{33} 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{14}

The mechanism involved in the AA-induced growth retardation is not clear. Prostaglandins of the E series suppress food intake\textsuperscript{29} probably through interference with hypothalamic regulation of food intake.\textsuperscript{33} However, other mechanisms, e.g., reset of metabolic rate or suppressed food intake\textsuperscript{32} probably through interference with hypothalamic regulation of food intake, 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.

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