Antihypertensive Effect of \(\gamma\)-Linolenic Acid in Spontaneously Hypertensive Rats

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The effects of chronic treatments of adult (aged 16–17 weeks) spontaneously hypertensive rats (SHRs) with different doses of \(\gamma\)-linolenic acid (GLA) on blood pressure, heart rate, and body weight were studied. Twice-daily injection of SHRs with GLA lowered systolic blood pressure from 175±4 to 145±4 mm Hg within 1 week; systolic blood pressure in all three treated groups became stabilized in the normotensive range after 2 weeks of treatment. Control SHRs injected with olive oil showed only a transient decrease in systolic blood pressure on the third day. Heart rate and body weight were not affected by GLA treatment. Withdrawal of GLA treatment resulted in a rapid rise in systolic blood pressure within 1 day from 140±3 to 165±3 mm Hg, and it stabilized after 1 week at 191±5 mm Hg in the three experimental groups. A rapid increase in systolic blood pressure from 175±5 to 203±5 mm Hg was also observed in the control group treated with olive oil 1 day after the withdrawal of the treatment. Addition of aspirin (3 mg/kg) with the GLA treatment in olive oil abolished the antihypertensive effect of GLA. In contrast, once-daily treatment with GLA also lowered systolic blood pressure of the SHR, but blood pressure was still in the hypertensive range (170±6 mm Hg). Systolic blood pressure of control SHRs treated with olive oil was not affected. Plasma from untreated SHRs contained a small amount of GLA. One hour after the injection, the plasma level of GLA increased. We conclude that GLA when given twice daily is an effective antihypertensive agent in the SHR. (Hypertension 1992;19[suppl II]:II-111-II-115)

Studies in humans and animal models have suggested a potential role for dietary lipids as a cause of hypertension.\(^1\) Diets containing high levels of polyunsaturated fat elicit a hypotensive effect over time, whereas high saturated fat feeding elevates blood pressure (BP) in animal models.\(^1\) \(\gamma\)-Linolenic acid (18:3n-6, GLA) is an essential fatty acid that can be readily prepared from evening primrose oil and used as a dietary fat. It belongs to a group of polyunsaturated omega-6 or n-6 essential fatty acids.\(^2\) Previous studies have shown that GLA could lower BP in stress-induced hypertensive rats\(^3\) and attenuate the development of hypertension in the spontaneously hypertensive rat (SHR) when treatment was initiated in young, prehypertensive rats.\(^4,5\) However, studies also showed that GLA alone did not alter baseline BP in borderline hypertensive rats (hybrids of SHRs and normotensive Wistar-Kyoto rats) but evening primrose oil did.\(^6\) Evening primrose oil is rich in n-6 fatty acids, with nearly 75% of its content composed of linoleic acid and approximately 9% of GLA.\(^3\) Linoleic acid can be converted to GLA by \(\Delta6\)-dehydrogenase and, through further elongation and desaturation, to arachidonic acid, which is a precursor of prostaglandins and leukotrienes.\(^7\)

The purpose of this study was to evaluate the effects of GLA on arterial BP in adult SHRs. In addition, we also investigated the time course of GLA plasma concentration to develop a rational approach to a dosing regimen.

Methods

Animals

Male SHRs were used and obtained from the colony maintained at the Animal Quarters of McMaster University, which originated from Charles Rivers Breeding Farm. They were fed standard Purina rat chow and tap water ad libitum.

Twice-Daily Treatment Study

Twelve- to 13-week-old SHRs were conditioned to handling and BP measurements for 4 weeks before the study. These rats were divided into four treatment groups. The three experimental groups (four
animals per group) were given intraperitoneal injections twice daily at 12-hour intervals for 6 weeks at dosages of 1.2 (group 1), 3.6 (group 2), and 7.2 (group 3) mg/kg/injection of GLA in olive oil. GLA was obtained through the generous donation of Biofarm, Ontario, Canada. Gas chromatography analysis of the GLA demonstrated purity of more than 95%. The concentration of GLA in olive oil was prepared such that for each rat, the total volume injected was maintained constant at 1.5 ml/kg for each injection. The control group received an equivalent amount of olive oil through injection.

Systolic BP and heart rate were measured by indirect measurement through the tail 1 hour after GLA injection, every 24 hours between 8 AM and 11 AM for the first week, and once a week for the next 5 weeks. Body weight was measured every other day. At the end of the 6-week treatment period, treatment of the rats was stopped. BP, heart rate, and body weight measurements were taken 24 hours before the termination of treatment and every other day for 2 weeks.

Once-Daily Treatment Study

Male SHRs 24–25 weeks old were divided into one control and three treatment groups (three animals per group). These rats were subjected to a once-daily intraperitoneal injection with either olive oil (control) or with one of the following dosages of GLA: 2.4 (group 1), 7.2 (group 2), or 14.4 (group 3) mg/kg/day. Total volume per injection again was maintained at 1.5 ml/kg. BP, heart rate, and body weight measurements were taken 24 hours before the beginning of the treatment and on the first, second, fifth, seventh, and 14th day during the 2-week treatment period.

Treatment of Spontaneously Hypertensive Rats With Acetylsalicylic Acid

Twelve male SHRs 24–22 weeks old were divided into three groups (four per group). Their BP, heart rate, and body weight were assessed for 1 week before the start of the experiments. One group received 14.8 mg/kg/day of GLA in olive oil; the second group received 3.0 mg/kg/day of acetylsalicylic acid (ASA); and the third group received a combination of 3.0 mg/kg/day ASA and 14.4 mg/kg/day GLA. Intraperitoneal injections were given twice daily at 12-hour intervals at a volume of 1.5 ml/kg/injection. BP, heart rate, and body weight were measured 24 hours before and after the beginning of the injections and subsequently on days 4, 7, and 14 of the experiments. ASA (Sigma Chemical Co., St. Louis, Mo.) was dissolved in 2.37 M sodium carbonate (100 mg/ml) with constant stirring to reach a pH of 7.0 or less. This was critical, because our high-performance liquid chromatographic analyses showed that at pH higher than 7.0, ASA was converted to acetylsalicylate.

Plasma concentration of GLA was determined by gas chromatography using the method of Rosenfeld et al. Blood was collected from an anesthetized (sodium pentobarbital, 35 mg/kg) adult SHR through a cannula inserted into the abdominal aorta. Plasma from this sample was used to construct a standard curve with known concentrations of added GLA. The standard curve showed a linear correlation between the amount of GLA added to plasma and the gas chromatographic response ($r=0.9742$, $p<0.03$).

To measure the plasma levels of GLA at different times after injection of GLA, we collected plasma samples in five conscious adult SHRs (24 weeks old) through a tail vein before and at 1 and 2 hours after intraperitoneal injection with 14.4 mg/kg GLA. An average of 0.5 ml plasma was obtained from each rat at each sampling. Fifty microliters of plasma was added to a 16x100-mm screw-cap silanized glass vial containing 300 mg XAD-2 resin (a styrene/divinylbenzene cross-linked copolymeric resin) and 4 ml of 0.1 M phosphate buffer solution at pH 7.4. The reaction was then initiated by adding 15 μl pentafluorobenzyl bromide in 135 μl diluent (tetrachloroethylen) and shaking the reaction mixture at ambient temperature for 20 minutes. The resin was isolated by filtration through a SupelClean cartridge fitted with a sintered disk and a Luer lock fitting. The reactor bed was washed with water until the aspirate was clear, and the resin was dried in vacuo.

The derivatized analyte was eluted from the resin by washing twice with 5 ml hexane. After the evaporation of the solvent, the residue was reconstituted in 400 μl toluene. The derivatives were determined using capillary column gas chromatography with electron-capture detection.

Statistics

Results are expressed as mean±SEM. Statistical analysis was made by one-way analysis of variance, by multiple $t$ test for comparison of the three means, and by Student’s $t$ test for comparison of two means. Values of $p<0.05$ were considered significant.

Results

Figure 1 shows systolic BP response to twice-daily treatment with different doses of GLA. The acute antihypertensive effect of GLA was already evident on the third day of treatment in all of the treated groups (Figure 1, top panel). By day 5 of treatment, systolic BP of the three treated groups had decreased from 175±4.0 mm Hg before treatment to a normotensive level (140±2 mm Hg). Systolic BP of the three GLA-treated groups became stabilized at around 140±1 mm Hg after 2 weeks and remained at that level during the following 5 weeks (Figure 1, bottom panel; Table 1). Systolic BP in control SHRs injected with olive oil was also lowered on the third day after the initiation of treatment, but the decrease
was transient, because the systolic BP had returned to the pretreatment level by day 5 (Figure 1, top panel). Heart rate was not affected in any of the four groups of rats (Table 1). Body weight increased steadily, and there was no difference in body weight among the four groups of rats.

Withdrawal of treatment caused a rapid increase in systolic BP in all four groups of rats after the first day, as shown in Figure 2. Systolic BP in the three GLA groups continued to rise subsequently and reached the level of the control group after 1 week (Table 1).

To determine whether systolic BP of the rats fluctuated between treatments, we measured systolic BP twice a day (once in the morning and again in the evening) 10 hours after injection with olive oil (control) or GLA (groups 1–3) for 1 week, 3 weeks after the initiation of treatment. We found that systolic BP remained fairly constant throughout the day, suggesting a prolonged antihypertensive effect of GLA.

Figure 3 shows systolic BP response to once-daily treatment. Systolic BP was lowered gradually during the first week of treatment from the pretreatment level of greater than or equal to 190 mm Hg and became stabilized during the second week, but the systolic BP of all the GLA-treated groups was still in the hypertensive range (Table 1). In contrast, treatment with olive oil did not have any effect on systolic BP. Heart rate and body weight of the rats were also not affected by treatment.

Figure 4 shows the effect of ASA on the systolic BP of SHRs. Treatment with GLA in olive oil lowered the BP of SHRs gradually, and BP became stabilized after 1 week and remained in the normotensive range (144±10 mm Hg) after 2 weeks of treatment, as observed before. Systolic BP of SHRs treated with ASA alone remained in the range of 180 mm Hg, being 181±4 mm Hg before and 184±7 mm Hg after 2 weeks of treatment. A combination of ASA and GLA treatments prevented the antihypertensive effect of GLA. Systolic BP of the SHRs continued to rise, reaching 201±7 mm Hg at the end of 2 weeks. The systolic BP of SHRs treated with ASA alone was lowered transiently after 1 week of treatment as compared with those rats treated with a combination of ASA and GLA, but the difference disappeared at the end of 2 weeks (Figure 4).

The basal concentration of GLA in the plasma was 7.55±0.45 μg/ml. In four of five animals, the plasma level of GLA increased to 9.19±0.93 μg/ml (22% increase, p=0.03 as compared with basal level before injection) 1 hour after injection. However, 2 hours after injection, the plasma level of GLA had returned to 8.40±1.52 μg/ml (p=0.44 as compared with basal level). In one animal, the increase in the plasma level of GLA occurred at 2 hours after injection (33% increase, p=0.002).

Discussion

The major findings of this study were that twice-daily treatment with GLA can attenuate systolic BP responses in the SHR and that normotension can be achieved within 1 week even with the lowest dose of GLA. This is in contrast to a previous study in which a similar dose of GLA (approximately 1.1–2.4 mg/kg/day) was found to have no effect on resting BP in Sprague-Dawley rats and borderline hypertensive rats, even though BP response to stress due to isolation and salt loading was attenuated. The discrepancy between the two studies in response to GLA during resting conditions probably reflects the
## Table 1. Physical Characteristics of Control Spontaneously Hypertensive Rats Treated With Olive Oil and Those Treated With Different Concentrations of γ-Linolenic Acid (Groups 1–3)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pretreatment</th>
<th>Week 1</th>
<th>Week 6</th>
<th>After withdrawal</th>
<th>Once-daily treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>169 ± 5</td>
<td>175 ± 6</td>
<td>175 ± 4</td>
<td>199 ± 5*</td>
<td>200 ± 4</td>
</tr>
<tr>
<td>Group 1</td>
<td>174 ± 4</td>
<td>145 ± 2†</td>
<td>142 ± 3†</td>
<td>200 ± 4*</td>
<td>182 ± 7</td>
</tr>
<tr>
<td>Group 2</td>
<td>178 ± 3</td>
<td>146 ± 2†</td>
<td>148 ± 3†</td>
<td>199 ± 3*</td>
<td>176 ± 5‡</td>
</tr>
<tr>
<td>Group 3</td>
<td>175 ± 7</td>
<td>143 ± 4†</td>
<td>144 ± 2†</td>
<td>190 ± 7*</td>
<td>170 ± 7‡</td>
</tr>
<tr>
<td>p (ANOVA)</td>
<td>0.66</td>
<td>0.0003</td>
<td>0.0001</td>
<td>0.47</td>
<td>0.01</td>
</tr>
<tr>
<td>Heart rate (min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>386 ± 10</td>
<td>365 ± 3</td>
<td>371 ± 4</td>
<td>354 ± 8</td>
<td>360 ± 12</td>
</tr>
<tr>
<td>Group 1</td>
<td>389 ± 12</td>
<td>389 ± 3</td>
<td>364 ± 3</td>
<td>392 ± 11</td>
<td>364 ± 3</td>
</tr>
<tr>
<td>Group 2</td>
<td>390 ± 13</td>
<td>373 ± 6</td>
<td>363 ± 5</td>
<td>384 ± 22</td>
<td>363 ± 11</td>
</tr>
<tr>
<td>Group 3</td>
<td>390 ± 14</td>
<td>353 ± 14</td>
<td>356 ± 9</td>
<td>384 ± 6</td>
<td>360 ± 17</td>
</tr>
<tr>
<td>p (ANOVA)</td>
<td>&gt;0.8</td>
<td>0.42</td>
<td>0.35</td>
<td>0.24</td>
<td>&gt;0.8</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>272 ± 12</td>
<td>282 ± 10</td>
<td>322 ± 12</td>
<td>348 ± 10</td>
<td>352 ± 10</td>
</tr>
<tr>
<td>Group 1</td>
<td>264 ± 4</td>
<td>280 ± 2</td>
<td>316 ± 4</td>
<td>356 ± 3</td>
<td>353 ± 6</td>
</tr>
<tr>
<td>Group 2</td>
<td>272 ± 1</td>
<td>280 ± 4</td>
<td>313 ± 2</td>
<td>342 ± 2</td>
<td>342 ± 5</td>
</tr>
<tr>
<td>Group 3</td>
<td>269 ± 24</td>
<td>279 ± 24</td>
<td>306 ± 25</td>
<td>330 ± 24</td>
<td>319 ± 21</td>
</tr>
<tr>
<td>p (ANOVA)</td>
<td>&gt;0.8</td>
<td>&gt;0.8</td>
<td>&gt;0.8</td>
<td>0.58</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n=4 in each group. ANOVA, analysis of variance.  
*p<0.01 as compared with week 6.  
†p<0.005 as compared with control.  
‡p<0.01 as compared with after withdrawal.

genetic differences in fatty acid metabolism and BP regulation among different rat strains.  
Because we were using bolus intraperitoneal injection of GLA in our protocol, we were concerned about the duration of the GLA effect on BP. We have found that GLA can maintain BP within the normotensive range even up to 10 hours after injection. To our knowledge, this study constitutes the first report showing that GLA can control BP in the SHR for at least 10 hours after injection. In contrast, once-daily treatment of SHRs with GLA did not reduce BP to a normotensive level. It is possible that GLA was metabolized by the SHR within 24 hours so that its effect on systolic BP did not extend beyond 24 hours.

The mechanism of action of GLA in BP regulation is largely unknown. One possible explanation is that GLA administration might increase dihomo-GLA or arachidonic acid stores in the basal condition so that under conditions of stimulated prostaglandin synthesis (e.g., stress), there is a greater production of various prostaglandins, such as prostaglandin E and I series, which are predominantly vasodilatory in function. Mills and Ward suggested that increased prostaglandin E biosynthesis from GLA was absent in unstressed animals because of their negative findings in Sprague-Dawley rats. Hoffmann et al also suggested that production of vasodilator prostaglandins may not be the only mechanism, because they
have found that even though chronic treatment of young SHRs with GLA for 14 weeks through dietary means increased the tissue levels of prostaglandin I₂, there was a greater production of the potent vasoconstrictor thromboxane A₂. Our results suggest that prostaglandin synthesis might be augmented and was responsible for the antihypertensive property of GLA treatment, because inclusion of ASA with the GLA treatment was effective in abolishing the antihypertensive effect of GLA in adult SHRs. Other proposed mechanisms of action of GLA include suppression of sympathetic activity and decreased reactivity to pressor agents such as circulating catecholamines and angiotensin.⁴,⁶

Mills et al.⁵ argued that olive oil did not have any effect on either systolic BP or heart rate. Our experiments showed that olive oil actually had a slight antihypertensive effect on the SHR. A possible explanation for this effect is that olive oil contains high levels of oleic acid (18:1n-9, 76%) and linoleic acid (9%).⁶ Both can be converted to GLA through the oxidative actions of dehydrogenases, so the levels of GLA could be augmented by the use of olive oil. Generally, twice- or once-daily treatment with GLA did not affect the heart rate and growth of SHRs. Previous studies in borderline hypertensive rats also showed that GLA had no effect on heart rate but may retard body weight gain.⁶

In our experiments, we have found that there was a basal level of GLA in the blood. We also found that the time course of plasma level of GLA after intraperitoneal injection was consistent with concepts of variable tissue absorption of GLA and a short plasma half-life of injected GLA. It is possible that the action of GLA is at the level of the cell membrane rather than in the blood circulation. The prolonged antihypertensive action of GLA awaits further investigation.

In conclusion, we have shown that GLA was very effective in reducing systolic BP in the SHR. The best response was obtained in a twice-daily regimen rather than a once-daily treatment.

**Acknowledgment**

We thank Sheila Seaman for her secretarial assistance.

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**KEY WORDS** • essential hypertension • linoleic acids • antihypertensive therapy • spontaneously hypertensive rats
Antihypertensive effect of gamma-linolenic acid in spontaneously hypertensive rats.
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Hypertension. 1992;19:II111
doi: 10.1161/01.HYP.19.2_Suppl.II111

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