Persistent Reduction in Renal Nerve Growth Factor mRNA After Perindopril Treatment of Young Spontaneously Hypertensive Rats

Fadi J. Charchar, Miroslav Kapuscinski, Stephen B. Harrap

Abstract—Nerve growth factor (NGF) determines sympathetic innervation of target tissues, and NGF levels are increased in young spontaneously hypertensive rats (SHR). Angiotensin can affect NGF levels, and the persistent reduction in blood pressure after brief angiotensin-converting enzyme inhibition in young SHR may involve long-term changes in NGF and sympathetic innervation. We measured the relative abundance of renal NGF mRNA by reverse transcription–polymerase chain reaction in SHR during and after treatment from 6 to 10 weeks of age with vehicle, perindopril (3 mg/kg per day), the bradykinin B2 antagonist Hoe 140 (0.5 mg/kg per day), both perindopril and Hoe 140, or angiotensin II (Ang II; 200 ng/kg per minute). Glomerular filtration rates were estimated at 10 and 20 weeks of age. At 10 weeks of age, Ang II caused a significant (P<.01) increase and perindopril caused a significant (P<.01) decrease in renal NGF mRNA levels. Blockade of the bradykinin B2 receptor during perindopril treatment attenuated (P<.05) the reduction in NGF mRNA levels. Renal NGF mRNA (P<.005) and blood pressure (P<.001) remained significantly lower than control 10 weeks after perindopril treatment was stopped. The partial reduction in blood pressure at 20 weeks of age in rats that had received perindopril and Hoe 140 was not associated with any difference in renal NGF mRNA. Perindopril-induced long-term reduction in renal NGF mRNA levels may decrease sympathetic innervation and thereby contribute to the long-term posttreatment blood pressure reduction. (Hypertension. 1998;31:678-683.)

Key Words: nerve growth factor ■ angiotensin-converting enzyme ■ sympathetic nervous system ■ bradykinin ■ genetics

The renin-angiotensin and sympathetic nervous systems contribute to the development of hypertension in the SHR. These two control systems also show significant interaction that may be relevant, particularly in the kidneys of young SHR, in which increased renin gene expression and heightened renal sympathetic nerve activity have been demonstrated.6,7

Treatment with the ACE inhibitor perindopril is followed by a long-term reduction in BP.1,8 Surgical ablation of the renal sympathetic nerves has also been shown to prevent the development of hypertension in young SHR.9 ACE inhibition may have both functional and structural effects on the sympathetic nerves. For example, angiotensin can enhance sympathetic nerve transmission and ACE inhibitors have sympatholytic effects.10 In addition, angiotensin may modulate sympathetic innervation by effects on NGF in key tissues. Angiotensin increases the secretion of NGF from vascular smooth muscle cells in vitro,11,12 and Ang II receptor blockade reduces tissue levels of NGF.13

In most tissues, including the kidney, the density of sympathetic innervation correlates closely with tissues levels of NGF peptide and mRNA that have been described in young SHR.16-21

Our hypothesis was that ACE inhibition, by reducing NGF in key tissues (ie, the kidneys) at a critical time (ie, in youth), would reduce renal sympathetic innervation in a long-term manner. In effect, early ACE inhibition would produce a partial pharmacological sympathectomy. To test this hypothesis, we measured renal NGF mRNA levels during and after treatment of young SHR with the ACE inhibitor perindopril. Renal levels of NGF mRNA have been shown to correlate with altered sympathetic innervation in SHR,22 and perindopril treatment causes a significant reduction in renal angiotensin peptide levels.23 Because the persistent reduction in BP after ACE inhibition may also be explained in part by the accumulation of bradykinin during treatment,24 the effect of bradykinin antagonism on renal NGF mRNA was also studied.

Methods

Animals

Four-week-old male SHR, originally derived from National Institutes of Health stock were obtained from an inbred colony in the Biological Research Laboratories, Austin Hospital, Melbourne, Australia. The SHR colony was subjected to regular tests with biochemical polymorphic markers to ensure its inbred status, which was confirmed

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Clearance was measured at 10 weeks of age in all animals using the single-shot radiolabeled DTPA method.26 A calibrated dose of technetium reduced with stannous chloride complexed to DTPA (Sigma Chemical Co) was injected into the tail vein of conscious rats. After 43 minutes, a blood sample was taken from a different tail vein and centrifuged in a cooled centrifuge and the supernatant was collected for counting. Radioactivity was counted in a gamma counter and compared with a reference prepared at the time of injection. 26 GFR was measured at 10 weeks of age in all animals using the single-shot radiolabeled DTPA method.26 A calibrated dose of technetium reduced with stannous chloride complexed to DTPA (Sigma Chemical Co) was injected into the tail vein of conscious rats. After 43 minutes, a blood sample was taken from a different tail vein and centrifuged in a cooled centrifuge and the supernatant was collected for counting. Radioactivity was counted in a gamma counter and compared with a reference prepared at the time of injection. 26

Ethics Committee.

ANCOVA was used to test differences in tail BP, body weight, and GFR between the different treatment groups in the short-term and long-term studies separately. Between-group differences were tested using ANCOVA with terms for treatment, study period, and study period by treatment interaction. The F-statistic was used to test the significance of the differences between treatment groups. The significance level was set at 0.05 for all analyses. All data are presented as mean±SEM unless stated otherwise.ANOVA was used to test differences in tail BP, body weight, and GFR between the different treatment groups in the short-term and long-term studies separately. Between-group differences were tested using ANCOVA with terms for treatment, study period, and study period by treatment interaction. The F-statistic was used to test the significance of the differences between treatment groups. The significance level was set at 0.05 for all analyses. All data are presented as mean±SEM unless stated otherwise.
TABLE 1. Average Abundance of Renal NGF mRNA at 10 and 20 Weeks of Age

<table>
<thead>
<tr>
<th>Age</th>
<th>Control</th>
<th>Hoe 140</th>
<th>Perindopril+Hoe 140</th>
<th>Perindopril</th>
<th>Ang II</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 Weeks</td>
<td>0.79±0.06</td>
<td>0.75±0.03†</td>
<td>0.66±0.06†</td>
<td>0.53±0.04*</td>
<td>0.90±0.04†</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>20 Weeks</td>
<td>0.60±0.08</td>
<td>0.61±0.07†</td>
<td>0.63±0.10†</td>
<td>0.46±0.03*</td>
<td></td>
<td>.005</td>
</tr>
</tbody>
</table>

Data are expressed as the ratio of fluorescence output NGF/fluorescence output GAPDH and corrected for background fluorescence.

*P<.05 compared with control SHR; †P<.05 compared with perindopril-treated SHR.

NGF mRNA Levels

Fig 2 shows representative images of NGF and GAPDH PCR products from kidney cDNA as scanned by the FluorImager. The sizes of PCR products were 440 bp for NGF and 220 bp for the GAPDH product. Southern blot analysis using oligonucleotide probe internal to the PCR primers (data not shown) confirmed the identity of these products. Relative abundance of renal NGF mRNA in the five treatment groups after correction for GAPDH is given in Table 1.

There were no significant differences between replicates for each animal as analyzed by repeated measures ANOVA (P=.39). The NGF mRNA levels were highest in the Ang II–treated rats (P<.01) and lowest for the perindopril-treated rats (P<.01) at 10 weeks of age compared with untreated SHR. Hoe 140 treatment had no significant effect on NGF mRNA levels. However, treatment with both Hoe 140 and perindopril resulted in an intermediate level of NGF mRNA significantly different from both control (P<.05) and perindopril–treated (P<.05) animals (Table 1). No correlation was observed between NGF mRNA levels and BP at 10 weeks of age in any group.

Renal Function

Table 2 shows the average GFR values, which were significantly different (P<.0001, ANOVA) between the treatment groups at 10 weeks of age. Perindopril–treated SHR showed significantly higher GFR values than any other group. The GFRs of animals receiving both Hoe 140 and perindopril, Hoe 140 alone, or Ang II were significantly lower than those of controls. No correlation was observed between NGF mRNA levels and GFR at 10 weeks of age in any group.

Long-term Study

The average weekly body weights of the four groups in the long-term study were not significantly different before, during, or after treatment. During treatment, the tail BPs showed changes similar to those seen in the short-term experiment (Fig 3). All rats showed an increase in BP in the 2 weeks after treatment was stopped, but the magnitude of increase varied between groups. Between 11 and 12 weeks of age, the tail BP of control animals rose on average by 7.5 mm Hg compared with an average rise of 39 mm Hg in the rats that had been treated with both Hoe 140 and perindopril. The pressure increases in the SHR treated with either perindopril or Hoe 140 were of a magnitude similar to that of the control group.

Results

Short-term Study

The average weekly body weights for each of the five groups in the short-term study revealed that before and during treatment all animals grew normally and there were no significant differences (P=.243). Average tail BP values are shown in Fig 1 for the short-term study. Control SHR showed a steady rise in BP, characteristic of the developmental phase of hypertension. Their average tail BP at 10 weeks of age was 193±5 mm Hg. Ang II–treated rats showed a significant increase in BP (P<.001, ANOVA) at 10 weeks of age with an average rise of 39 mm Hg in the rats that had been treated with both Ang II and Hoe 140. Hoe 140 alone produced no significant change in BP compared with the control rats (182±4 mm Hg). Perindopril treatment decreased BP significantly (P<.05 compared with perindopril-treated SHR). The average weekly body weights of the four groups in the long-term study were not significantly different before, during, or after treatment. During treatment, the tail BPs showed changes similar to those seen in the short-term experiment (Fig 3). All rats showed an increase in BP in the 2 weeks after treatment was stopped, but the magnitude of increase varied between groups. Between 11 and 12 weeks of age, the tail BP of control animals rose on average by 7.5 mm Hg compared with an average rise of 39 mm Hg in the rats that had been treated with both Hoe 140 and perindopril. The pressure increases in the SHR treated with either perindopril or Hoe 140 were of a magnitude similar to that of the control group.

![Figure 1.](Image)

Average biweekly tail blood pressures of five groups of male SHR between 6 and 10 weeks of age. Data are mean±SEM. Treatment between 6 and 10 weeks of age consisted of vehicle controls (lane 3), perindopril (lane 4), Hoe 140 (lane 5), perindopril plus Hoe 140 (lane 2), or Ang II (lane 1).

![Figure 2.](Image)

Representative images of the PCR products after gel electrophoresis and quantitative scanning by the FluorImager for the five treatment groups at 10 weeks of age. The first row shows the amplified NGF product at ~440 bp, the second row represents the GAPDH PCR product at ~220 bp. Treatment between 6 and 10 weeks of age consisted of Ang II (lane 1), Hoe 140 (lane 2), perindopril plus Hoe (lane 3), perindopril (lane 4), or vehicle controls (lane 5).
Between 13 and 20 weeks of age, the control SHR and Hoe 140–treated groups showed a steady rise in BP, although at a slower rate compared with that at 6 to 10 weeks of age. SHR treated with perindopril alone showed a very slow rise in BP between 13 and 20 weeks of age and at 20 weeks of age had a BP (average, 165 ± 1.1 mm Hg) significantly lower than all other groups (P < .0001, ANOVA). At 20 weeks of age, the tail BP of rats that had been treated with perindopril plus Hoe 140 (average, 189 ± 1.5 mm Hg) was significantly higher than that of the rats treated with perindopril alone but also significantly lower than that of the controls (average, 209 ± 3.0 mm Hg) and SHR that received Hoe 140 alone (average, 208 ± 1.6 mm Hg) (P < .0001, ANOVA).

NGF mRNA Levels

Compared with the levels seen at 10 weeks of age, the relative abundance of renal NGF mRNA in control SHR was significantly lower (P < .05 by independent t test) in the 20-week-old control SHR. That SHR, which received perindopril between 6 and 10 weeks of age showed significantly lower levels of NGF mRNA expression (P = .005, ANOVA) than the other three treatment groups (Table 1). Hoe 140 and perindopril plus Hoe 140 treatments showed no significant difference in NGF mRNA compared with the control group. No correlation was observed between NGF mRNA levels and BP at 20 weeks of age in any group.

Renal Function

At 20 weeks of age, the perindopril-treated rats showed the highest average GFR, but ANOVA revealed marginal statistical significance for this result (P = .055, ANOVA). Renal NGF mRNA levels did not correlate with GFR at 20 weeks of age.

**Discussion**

This study reveals that the reduction in BP that occurs during and after perindopril treatment in young SHR is associated with a significant reduction in the relative abundance of renal NGF mRNA. The decrease in NGF gene expression may be an important component in the short-term and long-term effects of ACE inhibition in this strain.

Previous studies have emphasized the potential importance of NGF in the development and maintenance of high BP in the SHR. Both NGF mRNA and peptide levels are significantly higher in SHR than in normotensive strains in key tissues, including the kidneys and resistance vessels. Because NGF levels have been shown to correlate closely with the degree of sympathetic innervation, it seems likely that NGF in SHR contributes to the increased sympathetic innervation and activity that are evident in histological, electrophysiological, and biochemical experiments. The prevention of hypertension by administration of anti-NGF antibodies to young SHR is also consistent with an etiologic role for NGF in this genetic model of hypertension.

Our results indicate that angiotensin exerts an important control over renal NGF expression. The relative abundance of NGF mRNA in young SHR was increased by angiotensin infusion and decreased by ACE inhibition. These findings extend previous results in other studies in which exogenous angiotensin increases NGF production, whereas AT1 angiotensin receptor antagonism decreases NGF levels.

The most interesting observation was that brief treatment with perindopril was followed by a persistent and significant reduction in renal NGF mRNA. The findings imply that perindopril in some way resets renal NGF gene expression in young animals and that this effect continues into adulthood. The consequences of such downregulation are likely to affect sympathetic innervation in the kidney. NGF plays a central role as a trophic signal from tissues to the sympathetic nerves. It is important particularly in the perinatal period, when levels of NGF determine the degree of neuronal apoptosis and thereby set the degree of sympathetic innervation of the target tissues. NGF is also important in maintaining sympathetic innervation of target tissues into adult life. Although direct studies of innervation were not performed in these experiments, the correlation among NGF mRNA and peptide, and sympathetic innervation in target tissues is well established. Therefore, it seems likely that brief ACE inhibition, by resetting renal NGF gene expression, may induce partial renal sympathectomy. This may explain the similarity in the BP effects of surgical renal denervation and brief ACE inhibition in young SHR.
The timing of ACE inhibitor treatment in relation to NGF effects may also be important. The results from this experiment show that the relative abundance of NGF mRNA was highest in young animals and decreased with age in untreated SHR. Similar findings have been reported by other investigators. It seems also that the increase in NGF occurs only in certain tissues. In young SHR, the kidneys, spleen, blood vessels, and sympathetic nerves, but not the heart, show high NGF levels. This tissue-specific and developmental stage-specific increase of NGF in SHR may not only establish high levels of sympathetic innervation but also define a window during which ACE inhibitor treatment has an effect that is perpetuated into later life.

Our study raises interesting questions about the control of NGF gene expression in the kidney. The explanation for developmental stage-specific changes of NGF in SHR is not known, but altered transcriptional control of the NGF gene may be the result of genetic mutation in gene regulatory regions. We have identified linkage between the NGF gene and the inheritance of high BP in genetic crosses of SHR, although functional mutations have not yet been identified. It is possible that mutations in other genes have an impact on NGF gene expression. For example, increased renal renin gene expression may modulate tissue angiotensin and raise NGF mRNA in young SHR.

In addition to the influence of angiotensin, our findings also indicate that bradykinin may be important, at least in the pharmacological actions of perindopril. The accumulation of bradykinin during treatment may contribute to the reduction of renal NGF mRNA. Blockade of the bradykinin B2 receptor with Hoe 140 during perindopril treatment significantly attenuated the reduction of NGF mRNA observed with perindopril alone. However, the role of bradykinin may not be relevant to normal physiology because the administration of Hoe 140 alone had no significant effect on basal SHR NGF mRNA levels.

Interestingly, the effects of bradykinin accumulation on NGF mRNA appear relevant only to the treatment period and not to the long-term resetting of renal NGF mRNA. This is in contrast with the BP effects, in which bradykinin does not appear to contribute to lower pressure during perindopril treatment but is partially responsible for the long-term reduction in pressure after perindopril. Clearly, the mechanisms of BP reduction differ during and after treatment. Notably, the partial reduction in long-term BP in rats that had received perindopril and Hoe 140 was not associated with any change in renal NGF mRNA. Presumably, not all of the long-term effects of ACE inhibitors are related to changes in renal NGF. However, our findings suggest that the additional pressure reduction observed in the perindopril-treated rats is related to the lower NGF mRNA.

Given that Ang II increases and perindopril decreases both BP and NGF mRNA, it could be argued that changes in NGF mRNA are simply the result of changes in BP. However, our results argue against such a generality. First, at 10 weeks of age, SHR receiving perindopril plus Hoe 140 had the same BP as the same animals treated with perindopril alone. Second, at 20 weeks of age, SHR that had been treated with both perindopril and Hoe 140 had the same NGF mRNA levels but significantly lower BPs than control SHR. Third, there was no significant correlation in any individual group between renal NGF mRNA and BP. Finally, the increase in BP observed in untreated SHR was accompanied by a fall in renal NGF mRNA.

We have described previously that perindopril treatment is associated with an increase in GFR in young SHR. The present study demonstrated significantly elevated GFR at 10 weeks but no significant change in the long term, possibly because of a shorter ACE inhibitor treatment period. Although we observed opposite changes in NGF mRNA and GFR in SHR treated with perindopril and Ang II, there was no correlation between renal NGF mRNA and GFR. The significant changes in NGF mRNA levels in the absence of GFR alteration in the SHR receiving perindopril plus Hoe 140 also indicates that the link between these renal molecular and functional characteristics is not absolute.

In summary, these studies raise some interesting questions regarding ACE inhibition and the expression of the NGF gene in SHR. They also indicate the importance of treatment at an early age and its long-term effect on adult renal NGF gene expression and BP.

Acknowledgments

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References

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