Elevated Fos Expression in the Nucleus Tractus Solitarii Is Associated With Reduced Baroreflex Response in Spontaneously Hypertensive Rats


Abstract—We delineated the functional role of Fos protein at the nucleus tractus solitarii in the manifestation of reduced baroreceptor reflex control of heart rate during hypertension, using spontaneously hypertensive rats (SHR), stroke-prone SHR, Wistar-Kyoto rats, or Sprague-Dawley rats. Microinjection into the bilateral nucleus tractus solitarii of an antisense oligonucleotide that targets against the initiation codon of c-fos mRNA significantly potentiated the baroreceptor reflex in response to 30 minutes of sustained increase in blood pressure. Of particular note was the restoration of both the impaired sensitivity and capacity of baroreceptor reflex in SHR and stroke-prone SHR to levels comparable to those in normotensive rats. Likewise, the number of Fos-immunoreactive nuclei evoked by the sustained increase in blood pressure in the caudal nucleus tractus solitarii of SHR and stroke-prone SHR was reduced, after this antisense c-fos treatment, to the basal level exhibited by the normotensive animals. Control treatment with the corresponding sense oligonucleotide, an antisense oligonucleotide that targets against a different portion of the coding sequence of the c-fos mRNA or artificial cerebrospinal fluid, on the other hand, elicited no discernible effect on either the baroreceptor reflex response or the induced expression of Fos protein in the nucleus tractus solitarii by baroreceptor activation. We also found that the basal level of Fos expression in the caudal nucleus tractus solitarii was significantly elevated in the SHR and stroke-prone SHR. Together, these novel findings suggest that an elevated expression of basal Fos protein in the NTS during hypertension may be associated with the dysfunction in baroreceptor reflex control of heart rate. (Hypertension. 1998;32:939-944.)

Key Words: antisense elements • baroreflex • hypertension, genetic • rats

Maintenance of a stable blood pressure through the arterial baroreceptor reflex (BRR) is a fundamental operating mechanism in central cardiovascular regulation.1 As such, impairment of BRR control of heart rate (HR) has been demonstrated in hypertensive patients2,3 and animals with genetic4–6 or experimentally induced hypertension.7,8 In addition to alterations in the afferent limb of this reflex,9,10 abnormalities in the brain stem components of the reflex arc11,12 have been implicated as causes for BRR dysfunction in hypertension. These result in a rightward shift (resetting) of the blood pressure–heart rate curve, a decrease in reflex gain, and a reduction in maximal bradycardia.2,4–8,13 Since a reduced BRR response is common among hypertension with different etiologies, it has been suggested that deficits in the BRR control of HR are not genetically determined but are a consequence of hypertension.13 Nonetheless, the precise mechanisms that underlie these BRR abnormalities after the onset of hypertension are not fully understood.

The immediate early gene c-fos and its protein product, Fos, are now known to couple short-term transsynaptic events to long-term changes in cellular phenotype by regulation of gene expression in neurons.14,15 In this regard, an increase in systemic arterial pressure (SAP) induces the expression of Fos protein in the nucleus tractus solitarii (NTS),16–18 the principal recipient of primary baroreceptor afferent fibers in the brain stem.19 We further reported17,20 that Fos expression in the NTS represents an early intracellular event that leads to long-term inhibitory modulation of BRR response. Whether Fos protein in the NTS plays a role in the manifestation of the reduced BRR response during hypertension is hitherto unknown.

The present study was performed to assess the hypothesis that an enhanced expression of Fos protein in the NTS is associated with the reduced BRR response during chronic hypertension, with the use of normotensive and genetically hypertensive rats. Our fundamental strategy was to block Fos expression in the NTS with antisense oligonucleotide against c-fos mRNA and to examine the resultant alterations in BRR response, SAP, and HR. Our findings essentially validated the hypothesis and suggest a permissive role for c-fos gene at the NTS in the manifestation of a reduced BRR sensitivity during hypertension.

Received March 30, 1998; first decision April 13, 1998; revision accepted July 1, 1998.
From the Department of Education and Medical Research, Veterans General Hospital–Taipei (J.Y.H.C., W.C.C., H.Y.L.), and Center for Neuroscience, National Yang-Ming University (S.H.H.C.), Taipei, Taiwan, Republic of China.
Correspondence to Julie Y.H. Chan, PhD, Department of Education and Medical Research, Veterans General Hospital–Taipei, Taipei 11217, Taiwan, Republic of China. E-mail yhwa@vghtpe.gov.tw
© 1998 American Heart Association, Inc.
Hypertension is available at http://www.hypertensionaha.org

939
Methods

Animals

Experiments were performed in compliance with the *Guiding Principles in the Care and Use of Animals* endorsed by our institutional animal care committee. Male, adult (age, 14 to 20 weeks; weight, 230 to 300 g) normotensive Sprague-Dawley rats (SD), normotensive Wistar-Kyoto rats (WKY), or spontaneously hypertensive rats (SHR) or their stroke-prone substrains (SHRSP) were used.

General Preparation

Rats were anesthetized initially with pentobarbital sodium (40 mg/kg IP) to perform intubation of the trachea and cannulation of the right femoral artery and both femoral veins. Animals received thereafter a continuous infusion of pentobarbital sodium (10 to 15 mg/kg per hour IV). This management scheme was found to provide satisfactory anesthetic maintenance throughout the experiment while preserving the capacity of central cardiovascular regulation, including the BRR response.21

Pulsatile and mean systemic arterial pressure (MSAP), as well as HR, were recorded on a polygraph (Gould ES1000). Animals were mechanically ventilated to maintain the end-tidal CO₂ to be within 4% to 5%, as monitored by a capnograph (Datex Normocap). All data were collected from animals with a maintained rectal temperature of 37±0.5°C throughout the experiment.

Microinjection of Oligonucleotides into the NTS

Three 15-mer phosphorothioated c-fos oligonucleotides (Quality System, Taipei, Taiwan) were microinjected stereotaxically into the bilateral NTS as described previously.17,20,22,23 The key antisense oligonucleotide (ASON1) targets against a region of the c-fos mRNA that flanks the initiation codon (5'-129 to 143–3'). Our treatment controls included a sense c-fos oligonucleotide (SON) and an antisense oligonucleotide (ASON2) that targets against the initiation codon and a different portion of the coding sequence (5'-135 to 149–3') of the c-fos mRNA. Animals that were surgically prepared, placed in the stereotaxic headholder without subsequent experimental treatments, and maintained by intravenous infusion of pentobarbital for 120 minutes served as the sham control. Animals that received microinjection of artificial cerebrospinal fluid (aCSF) (pH 7.4) into the bilateral NTS served as the volume and vehicle control.

Evaluation of BRR Response

The arterial baroreceptors were activated by an increase in SAP evoked by infusion of phenylephrine for 30 minutes. Because of the difference in responsiveness to this vasoactive agent between normotensive and hypertensive rats, the rate of infusion was adjusted (5% to 12%, as monitored by a capnograph (Datex Normocap)). All data were collected from animals with a maintained rectal temperature of 37±0.5°C throughout the experiment.

Statistical Analysis

All values are expressed as mean±SEM. One-way or 2-way ANOVA was used, as appropriate, to assess the difference between experimental groups. This was followed by the Scheffé multiple range test for a posteriori comparison of individual means. 

Results

Effect of ASON1 Antisense c-fos Oligonucleotide Treatment on BRR Response

Based on the analysis of both reflex sensitivity and maximal bradycardic capacity in response to an increase in SAP, we found an enhancement in BRR response in SD, WKY, SHR, and SHRSP after microinjection into the bilateral NTS of c-olf nucleotide (ASON1, 50 pmol) directed against the c-fos mRNA. As shown in Table 1, the slope of individual regression lines that relate decreases in HR to increases in SAP was significantly increased after administration of ASON1 to the bilateral NTS. We noted, however, that similar to previous reports,4–6,10 this index of BRR sensitivity in SHR or SHRSP rats that received aCSF pretreatment, for example, was significantly lower than that detected in SD or WKY. When this difference is taken into consideration, it is immediately apparent that there was a much greater enhancement in the sensitivity of BRR control of HR in SHR or SHRSP than in SD or WKY. When this difference is taken into consideration, it is immediately apparent that there was a much greater enhancement in the sensitivity of BRR control of HR in SHR or SHRSP than in SD or WKY. However, the potentiation of BRR sensitivity by ASON1 treatment was significantly greater in SHR than in SHRSP (Table 1).

Compared with ASON1 control, the maximal reflex bradycardia in response to sustained increase in SAP was also significantly increased in SD, WKY, SHR, and SHRSP after microinjection of ASON1 into the bilateral NTS. Again, this indicated capacity of BRR control of HR was discernibly...
Effect of ASON1 Antisense c-fos Oligonucleotide Treatment on Fos-LI in the NTS Induced by Sustained Increase in Blood Pressure

Sustained increase in blood pressure also induced expression of Fos-LI in the NTS of SD, WKY, SHR, and SHRSP (Figure 2). Those Fos-positive neurons concentrated primarily at levels of the NTS that extended from 0.8 caudal to 0.2 rostral to the obex and exhibited similar topographic distribution patterns. Quantitative analysis also revealed no significant difference among these 4 strains of rats in the number of Fos-positive nuclei detected in the caudal NTS after sustained increase in SAP.

Twenty-four hours after microinjection into the bilateral NTS of ASON1, the Fos-LI normally induced in the NTS 120 minutes after sustained increase in SAP was markedly retarded in SD, WKY, SHR, and SHRSP (Figure 2). Such a decrease in Fos expression was localized primarily in the rostral part of the NTS. We ascertained that the appreciable decrease in Fos-LI at the NTS was not due to false-negative reactions, since Fos-LI was still demonstrated in the ventral lateral medulla in animals that received ASON1 antisense oligonucleotide treatment.

Effect of Control c-fos Oligonucleotides on BRR Response, SAP, and HR

We verified the specificity of the observed biological activity of ASON1 antisense c-fos oligonucleotide by evaluating the effects of 2 control sequences of oligonucleotide. Treatment with microinjection bilaterally into the caudal NTS of SD, WKY, SHR, and SHRSP (Table 2), although the respective SAP remained higher than that recorded from aCSF-treated SD or WKY. In contrast, ASON1 treatment elicited no significant effect on baseline SAP or HR in SD or WKY.
found that bilateral microinjection of the sense or ASON2 antisense c-fos oligonucleotide into the NTS produced minimal effect on SAP or HR in SD, WKY, SHR, or SHRSP (Table 2).

### Effect of Control c-fos Oligonucleotides on Fos-LI in the NTS Induced by Sustained Increase in Blood Pressure

Treatments with microinjection bilaterally into the caudal NTS of sense c-fos oligonucleotide resulted in no discernible effect on the number of Fos-positive cells in the NTS induced by sustained increase in SAP (Figure 3) in SD, WKY, SHR, or SHRSP. Similar observations were made in animals that received treatment with ASON2 antisense cDNA (Figure 3).

### Fos-LI in the NTS of Saline- and Sham-Control Animals

Intravenous infusion of saline alone resulted in much less expression of Fos-LI in the NTS (Figure 2) that was not topographically distributed. Intriguingly, quantitative analysis revealed the number of Fos-positive nuclei detected in the NTS of SHR and SHRSP animals was appreciably higher than that found in SD and WKY (Figure 2). In the sham-control groups in which rats were maintained under pentobarbital anesthesia and received surgical operation alone, Fos-LI was scarce and distributed sporadically among different levels of the NTS. Nonetheless, the average number of Fos-positive nuclei detected per section in SD (3.7 ± 0.5, n = 2) and WKY (4.2 ± 0.8, n = 2) was still discernibly less than that found in SHR (13.5 ± 2.1, n = 3) and SHRSP (12.7 ± 2.9, n = 3).

### Discussion

A novel finding in the present study was the contribution of an elevated basal expression of Fos protein in the NTS to the retarded BRR sensitivity detected in SHR and SHRSP. As stated earlier in this report, it is generally accepted that hypertension is associated with an impairment of BRR.

---

**Table 2. Basal MSAP or HR in Sham-Control SD, WKY, SHR, or SHRSP and in Animals After Microinjection of aCSF (50 nL), ASON1 (50 pmol), ASON2 (50 pmol), or SON (50 pmol) into Bilateral NTS**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>MSAP, mm Hg</th>
<th>HR, bpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>4</td>
<td>114±6</td>
<td>383±15</td>
</tr>
<tr>
<td>aCSF</td>
<td>5</td>
<td>116±4</td>
<td>389±18</td>
</tr>
<tr>
<td>ASON1</td>
<td>6</td>
<td>117±6</td>
<td>392±20</td>
</tr>
<tr>
<td>ASON2</td>
<td>6</td>
<td>120±4</td>
<td>401±17</td>
</tr>
<tr>
<td>SON</td>
<td>5</td>
<td>117±5</td>
<td>394±15</td>
</tr>
<tr>
<td>WKY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>4</td>
<td>107±5</td>
<td>392±9</td>
</tr>
<tr>
<td>aCSF</td>
<td>4</td>
<td>105±5</td>
<td>391±15</td>
</tr>
<tr>
<td>ASON1</td>
<td>5</td>
<td>109±6</td>
<td>395±13</td>
</tr>
<tr>
<td>ASON2</td>
<td>5</td>
<td>114±7</td>
<td>397±15</td>
</tr>
<tr>
<td>SON</td>
<td>4</td>
<td>109±6</td>
<td>396±10</td>
</tr>
<tr>
<td>SHR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>4</td>
<td>158±4†</td>
<td>375±8</td>
</tr>
<tr>
<td>aCSF</td>
<td>6</td>
<td>153±6†</td>
<td>364±10</td>
</tr>
<tr>
<td>ASON1</td>
<td>6</td>
<td>136±4†</td>
<td>307±16†</td>
</tr>
<tr>
<td>ASON2</td>
<td>5</td>
<td>156±5†</td>
<td>377±11</td>
</tr>
<tr>
<td>SON</td>
<td>4</td>
<td>157±6†</td>
<td>362±16</td>
</tr>
<tr>
<td>SHRSP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>4</td>
<td>162±4†</td>
<td>369±11</td>
</tr>
<tr>
<td>aCSF</td>
<td>5</td>
<td>163±8†</td>
<td>360±17</td>
</tr>
<tr>
<td>ASON1</td>
<td>6</td>
<td>143±6†</td>
<td>300±15†</td>
</tr>
<tr>
<td>ASON2</td>
<td>6</td>
<td>157±4†</td>
<td>355±20</td>
</tr>
<tr>
<td>SON</td>
<td>5</td>
<td>163±5†</td>
<td>363±13</td>
</tr>
</tbody>
</table>

Values are mean±SEM of MSAP and HR. n = number of animals per group. Results were obtained 24 hours after microinjection of aCSF or various oligonucleotides into the bilateral NTS.

*P<0.05 vs aCSF treatment within each strain.
†P<0.05 vs WKY that received the same pretreatments in the Scheffé multiple range analysis.

Figure 2. Distribution of Fos-LI in 8 representative rostral-caudal sections of the caudal NTS in SD, WKY, SHR, or SHRSP that received microinjection of aCSF or ASON1 (50 nmol) into the bilateral NTS 24 hours before 30-minute intravenous infusion of either saline or phenylephrine ([ ] BP). Values are mean±SEM; n = 4 to 6 animals per group. *P<0.05 vs the corresponding vehicle control in each strain; †P<0.05 vs WKY that received the same pretreatment in the Scheffé multiple range analysis.
control of HR,2–8 which is likely to be a consequence of the rise in SAP rather than a preexisting condition.13 In addition, this impairment is already present during the early phase of hypertension,24,25 when chronic structural changes in the heart, vasculature, and aorta or carotid sinus6–10,24,25 are not yet fully developed. These observations imply that functional alterations in the central component of the reflex loop are the primary contributor to BRR impairment during hypertension.11,12 By relating the enhanced induction of c-fos gene in NTS neurons to the reduction in BRR response in hypertensive rats, the present study identified that one of these functional alterations may be an elevated basal level of Fos protein in the NTS of all strains of animals studied. These Fos-positive neurons have been demonstrated to represent second-order neurons in the BRR pathways.10,27 Detailed examination revealed that whereas there was an ≈3-fold increase in the number of Fos-positive NTS neurons in normotensive rats, a 2-fold increase was detected in the hypertensive animals (Figure 2). These findings are in agreement with previous reports11,12,28 in which a deficit in synaptic transmission of baroreceptor afferents at the NTS has been put forth as a possible mechanism. Our present results further indicate that such a difference in the evoked response may also arise from an elevated basal Fos expression in hypertensive rats. This notion, however, is at variance with a recent report29 that basal Fos immunoreactivity in the NTS is similar between WKY and SHR. The difference in anesthetic agents used may account for such a discrepancy. As noted,29 fentanyl/midazolam used in their study depresses the constitutive increase in Fos activity in SHR. Furthermore, pentobarbital sodium reportedly exerts much less suppressive effect on Fos immunoreactivity than fentanyl/midazolam.29 Several pieces of evidence validate the significantly greater number of Fos-positive cells demonstrated in the NTS of hypertensive rats under basal physiological conditions. We detected comparable quantity of Fos-positive neurons in both sham-operated controls and SHR that received an infusion of saline. We also found that the enhanced basal expression of Fos protein in the NTS could be reversed to levels comparable to that in normotensive rats by microinjection of ASON1 into the bilateral NTS. Moreover, the same treatment restored the reduced BRR response in SHR and SHRSP animals to levels not different from those in normotensive rats.

Two studies30,31 in which antihypertensive agents were used to treat hypertension indicate that reduction in SAP by itself may directly potentiate the function of BRR. As such, it is possible that restoration of BRR sensitivity by ASON1 in SHR and SHRSP may result from the depressor effect of this antihypertensive.30,31 Possible that restoration of BRR sensitivity by ASON1 in SHR and SHRSP may result from the depressor effect of this antihypertensive agent. This possibility, however, is deemed unlikely since ASON1 treatment in SD and WKY potentiated BRR response without significantly affecting basal SAP and HR. Furthermore, bilateral application of ASON1 to the NTS elicited greater potentiation of BRR response in SHR than SHRSP (Table 1) while producing similar degrees of hypotension and bradycardia (Table 2).

The method we used to evaluate the sensitivity and capacity of BRR response is based on the activation of arterial baroreceptors by an increase in SAP induced by infusion of phenylephrine. To minimize the possibility of differential activation of the baroreceptors due to differences in the vascular responsiveness among the 4 strains of animals to this vasoactive agent, the rate of infusion was adjusted to maintain a similar increase in SAP. Since phenylephrine does not cross the blood-brain barrier,32 it is unlikely that the difference in total doses of phenylephrine that normotensive or hypertensive rats received was a potential confounder that affected the BRR sensitivity. We also acknowledge that by long-term retardation of the BRR response in SHR and SHRSP.

Parallel to a recent study,26 we found that sustained increase in blood pressure evoked a significant increment in Fos-LI at the NTS of all strains of animals studied. These Fos-positive neurons have been demonstrated to represent second-order neurons in the BRR pathways.10,27 Detailed examination revealed that whereas there was an ≈3-fold increase in the number of Fos-positive NTS neurons in normotensive rats, a 2-fold increase was detected in the hypertensive animals (Figure 2). These findings are in agreement with previous reports11,12,28 in which a deficit in synaptic transmission of baroreceptor afferents at the NTS has been put forth as a possible mechanism. Our present results further indicate that such a difference in the evoked response may also arise from an elevated basal Fos expression in hypertensive rats. This notion, however, is at variance with a recent report29 that basal Fos immunoreactivity in the NTS is similar between WKY and SHR. The difference in anesthetic agents used may account for such a discrepancy. As noted,29 fentanyl/midazolam used in their study depresses the constitutive increase in Fos activity in SHR. Furthermore, pentobarbital sodium reportedly exerts much less suppressive effect on Fos immunoreactivity than fentanyl/midazolam.29 Several pieces of evidence validate the significantly greater number of Fos-positive cells demonstrated in the NTS of hypertensive rats under basal physiological conditions. We detected comparable quantity of Fos-positive neurons in both sham-operated controls and SHR that received an infusion of saline. We also found that the enhanced basal expression of Fos protein in the NTS could be reversed to levels comparable to that in normotensive rats by microinjection of ASON1 into the bilateral NTS. Moreover, the same treatment restored the reduced BRR response in SHR and SHRSP animals to levels not different from those in normotensive rats.

Two studies30,31 in which antihypertensive agents were used to treat hypertension indicate that reduction in SAP by itself may directly potentiate the function of BRR. As such, it is possible that restoration of BRR sensitivity by ASON1 in SHR and SHRSP may result from the depressor effect of this antihypertensive.30,31 Possible that restoration of BRR sensitivity by ASON1 in SHR and SHRSP may result from the depressor effect of this antihypertensive agent. This possibility, however, is deemed unlikely since ASON1 treatment in SD and WKY potentiated BRR response without significantly affecting basal SAP and HR. Furthermore, bilateral application of ASON1 to the NTS elicited greater potentiation of BRR response in SHR than SHRSP (Table 1) while producing similar degrees of hypotension and bradycardia (Table 2).

The method we used to evaluate the sensitivity and capacity of BRR response is based on the activation of arterial baroreceptors by an increase in SAP induced by infusion of phenylephrine. To minimize the possibility of differential activation of the baroreceptors due to differences in the vascular responsiveness among the 4 strains of animals to this vasoactive agent, the rate of infusion was adjusted to maintain a similar increase in SAP. Since phenylephrine does not cross the blood-brain barrier,32 it is unlikely that the difference in total doses of phenylephrine that normotensive or hypertensive rats received was a potential confounder that affected the BRR sensitivity. We also acknowledge that by
excluding animals that exhibited a MSAP <90 mm Hg for SD (n=2) or WKY (n=1) or <150 mm Hg for SHR (n=2) or SHRSP (n=2) in our experiments, possible confounding effects of surgical preparation and anesthesia might be overlooked. However, since there was no differential exclusion secondary to blood pressure of rats based on strain, the impact of surgical preparation and anesthesia alone on our present results was considered nominal.

In conclusion, our results suggest that an elevated basal expression of Fos protein in the NTS may contribute to the retarded BRR sensitivity detected during chronic hypertension.

Acknowledgments
This study was supported by research grant VGH87–387 from the Veterans General Hospital–Taipei and grant NSC-86–2314-B075–001-M10 from the National Science Council, Taiwan, Republic of China (to Dr J.Y.H. Chan).

References
Elevated Fos Expression in the Nucleus Tractus Solitarii Is Associated With Reduced Baroreflex Response in Spontaneously Hypertensive Rats