Atrial Natriuretic Peptide Modulates Baroreceptor Reflex in Spontaneously Hypertensive Rat

Hongkui Jin, Ren-Hui Yang, David A. Calhoun, James Michael Wyss, and Suzanne Oparil

Our previous studies have suggested that atrial natriuretic peptide in the caudal nucleus tractus solitarii is involved in the centrally mediated regulation of blood pressure in the salt-sensitive spontaneously hypertensive rat (SHR). The current study tested the hypothesis that endogenous atrial natriuretic peptide in the caudal nucleus tractus solitarii participates in baroreceptor reflex control of heart rate in this hypertensive model. Salt-sensitive SHR and control Wistar-Kyoto (WKY) rats maintained on basal (1%) salt intake were studied. Arterial baroreceptor reflex-mediated changes in heart rate were recorded in conscious unrestrained rats during phenylephrine (5–40 μg · kg⁻¹ · min⁻¹ infusion; 30 minutes later, atrial natriuretic peptide (50 ng), monoclonal antibody to atrial natriuretic peptide (0.55 μg), purified mouse immunoglobulin G (0.55 μg), or artificial cerebrospinal fluid vehicle (50 nl) was microinjected into the caudal nucleus tractus solitarii. Phenylephrine infusion was then repeated and mean arterial pressure and heart rate were monitored as before. The slope of the heart rate/mean arterial pressure relation was significantly less (p<0.05) in the salt-sensitive SHR than in the WKY control, indicating that baroreceptor reflex control of heart rate was blunted in this hypertensive model. Microinjection of atrial natriuretic peptide into the caudal nucleus tractus solitarii further blunted (p<0.05) baroreceptor reflex control of heart rate in salt-sensitive SHR but not in WKY rats. In contrast, microinjection of the monoclonal antibody enhanced the sensitivity of baroreceptor reflex control of heart rate in salt-sensitive SHR but not in WKY rats. These data suggest that endogenous atrial natriuretic peptide in the caudal nucleus tractus solitarii modulates baroreceptor reflex control of heart rate in salt-sensitive SHR but not in WKY rats. (Hypertension 1992;20:374–379)

KEY WORDS • antibodies, monoclonal • natriuretic peptides, atrial • brain • microinjections • blood pressure • pressoreceptors

Several lines of evidence indicate that atrial natriuretic peptide (ANP) in both the peripheral circulation and the central nervous system is involved in the reflex control of sympathetic nerve activity. In an anesthetized normotensive rat preparation, systemic administration of ANP has been shown to reduce sympathetic tone by a mechanism that involves a vagal afferent pathway and is partially buffered by arterial baroreceptor reflexes. Intravenous administration of ANP to anesthetized normotensive rats with intact arterial baroreceptors resulted in decreased sympathetic outflow accompanied by reductions in blood pressure and heart rate (HR). Sinoaortic denervation exaggerated these responses to intravenous ANP, whereas vagal cooling to 0°C reversibly abolished the sympathoinhibitory effect of intravenous ANP, indicating that this reflexly mediated action was dependent on afferent C-fiber activity in the vagus nerves. Further, recent studies have shown that circulating ANP in doses that have little effect on either blood pressure or HR exerts a complex modulatory effect on arterial baroreceptor reflexes in conscious normotensive rats. ANP markedly potentiated reflex bradycardia but attenuated reflex tachycardia in response to intravenous boluses of phenylephrine and nitroprusside, respectively; it did not modify the pressor response to carotid occlusion. Mechanisms that have been added to account for these effects of ANP include sensitization of cardiopulmonary receptors with consequent activation of afferent vagal pathways; sensitization of baroreceptor afferents, resulting in an increase in their responsiveness during stimulation, a maintenance of their discharge during deactivation, and direct excitation of neurons in the nucleus tractus solitarii (NTS). Studies in which ANP was injected into the cerebroventricular system have given direct evidence that ANP can lower blood pressure and reduce sympathetic activity by a central mechanism. Injection of ANP into the third ventricle has a sympatholytic effect, decreasing nerve traffic in renal and least-splanchnic sympathetic nerves and lowering blood pressure and HR in sinoaortic-denervated, chloralose-urethane sodium-anesthetized Sprague-Dawley
rats. Experiments carried out in our own laboratory showed that injection of ANP into the third ventricle produced small but significant reductions in blood pressure and lumbar sympathetic nerve traffic in salt-sensitive spontaneously hypertensive rats (SHR-S) but not in salt-resistant SHR (SHR-R) or Wistar-Kyoto (WKY) rats. ANP and ANP receptors are located in NTS and in other central sites known to be involved in cardiovascular regulation. The caudal NTS (C-NTS) is the primary site of termination for carotid and aortic baroreceptor afferents and thus plays an important role in the central regulation of blood pressure and in the central control of baroreceptor reflex function. Microinjection of exogenous ANP into C-NTS has been shown to increase the firing rates of NTS neurons and to reduce arterial pressure in anesthetized Wistar rats. Further, single units excited by microinjection of ANP into C-NTS were also excited by activation of arterial baroreceptors and inhibited by baroreceptor unloading. These findings suggest that ANP-induced activation of NTS neurons may mediate the depressor effect associated with arterial baroreceptor reflex activation.

Previous studies from our own laboratory have demonstrated that microinjection of monoclonal antibody to ANP into the C-NTS produces significant increases in blood pressure in SHR-S and SHR-R but not in WKY rats. Antibody injections evoked similar increases in mean arterial pressure (MAP) in SHR-S on both 1% and 8% NaCl diets and in SHR-R on a 1% NaCl diet. Control injection of an equal volume of immunoglobulin G (IgG) into the C-NTS had no effect on blood pressure in any strain. Further, microinjection of the antibody into the adjacent hypoglossal nucleus, spinal trigeminal nucleus or cuneate nucleus did not significantly alter blood pressure. The data suggest that endogenous ANP in C-NTS mediates tonic control of blood pressure in both SHR-S and SHR-R but not in WKY rats, and that this effect is independent of the salt sensitivity of hypertension and of dietary salt intake.

The current study tested the hypothesis that ANP receptors in C-NTS are involved in baroreceptor reflex control of HR in SHR-S. We studied arterial baroreceptor reflex-mediated changes in heart rate during phenylephrine infusion before and after microinjection of ANP, monoclonal antibody to ANP, IgG purified from murine ascites fluid, or artificial cerebrospinal fluid (ACSF) vehicle into C-NTS of conscious unrestrained SHR-S and WKY rats. We found that microinjection of ANP into C-NTS blunted baroreceptor reflex control of HR, whereas microinjection of the antibody improved baroreceptor reflex control of HR in SHR-S but not in WKY rats, suggesting that ANP in C-NTS has a tonic influence on the central regulation of baroreceptor reflex-mediated HR control in SHR-S but not in WKY rats.

Methods

Male SHR-S and normotensive WKY control rats were obtained from Taconic Farms (IBU-3 colony, Germiston, N.Y.) at 9 weeks of age. All rats were maintained four per cage at constant humidity (65±5%), temperature (24±1°C), and light cycle (6 AM to 6 PM). Rats were provided a standard rat diet (5001, Ralston-Purina, Richmond, Ind.) containing 1% NaCl and free access to food and water throughout the study. Two days before the acute experiment, each rat was anesthetized with sodium pentobarbital (50 mg/kg i.p.) and cannulas (PE-10 fused with PE-50) were implanted into the abdominal aorta through the right femoral artery for measurement of arterial pressure and into the right femoral vein for intravenous phenylephrine infusion. The rat was then placed into a stereotaxic apparatus, the skin overlying the middle of the skull was incised, and a small hole was drilled through the appropriate portion of the skull. A guide cannula (26-gauge stainless steel tubing) was lowered to a position 2.0 mm dorsal to the C-NTS (anteroposterior, -4.8 to 5.1 mm from the interaural line; mediolateral, 0.5 mm; dorsoventral, 8.5 mm; incisor bar, 4.5 mm) as previously described. All cannulas were placed in the right side of brain, and thus all injections were made unilaterally. The guide cannula was fixed to the skull with stainless steel screws and fast polymerized cannula cement. A 32-gauge obturator (stainless steel wire) was inserted into the guide cannula after implantation.

Forty-eight hours after surgery, the arterial cannula was connected to a model CP-01 pressure transducer (Century Technology Company, Inglewood, Calif.) coupled to a polygraph (model 7, Grass Instruments Company, Quincy, Mass.). Mean arterial pressure (MAP) and heart rate (HR) were recorded continuously throughout the study. After a 30-minute stabilization period, incremental doses of phenylephrine (5–40 μg) (Sigma Chemical Company, St. Louis, Mo.) were infused through the femoral vein catheter to achieve a ramp increase in MAP over 4 to 5 minutes. MAP and HR were then allowed to return to baseline during a second 30-minute stabilization period. After this stabilization period, the obturator was removed from the guide cannula and replaced with an inner cannula (32-gauge stainless steel tubing) filled with the agent to be administered. The tip of the inner cannula extended 2 mm beyond the guide cannula. The inner cannula was attached to a 0.5-μl Hamilton syringe through tubing (PE-20) filled with ACSF. A small air bubble was made between the ACSF and the injection solution. After insertion of the inner cannula and the return of vital signs to baseline, each rat was subjected to microinjection of ANP (30 ng) (Sigma) in 50 nl ACSF or ACSF vehicle into the C-NTS. Seven minutes later, the phenylephrine infusion was repeated and MAP and HR were monitored as before. All experiments were carried out in conscious, freely moving rats.

In parallel experiments, effects of blocking endogenous ANP in C-NTS by microinjection of monoclonal antibody to ANP on baroreceptor reflex–mediated responses of HR to phenylephrine infusion were examined. Surgery, arterial and venous cannulation, brain cannula implantation, and phenylephrine infusion were performed as above, except that monoclonal antibody to ANP (Mab KY-ANP-II, 0.55 μg), which had been purified by the procedure outlined below, or control mouse IgG (0.55 μg) purified from ascites fluid was microinjected into the C-NTS.

At the conclusion of each experiment, 1% methylene blue solution (50 nl) was injected through the brain cannula. The rat was anesthetized with sodium pentobarbital (60 mg/kg i.p.), decapitated, and the cannula
was removed from the brain. The brain was removed from the skull and sectioned at 30 μm on a freezing microtome (Slec Medical Equipment Ltd., London, UK). Sections were mounted and stained with 1% thionin for verification of the microinjection site and for measurement of extent of spread of the dye.

The monoclonal antibody used in these studies was the high affinity antibody against the 28-amino acid form of ANP rat atrial natriuretic factor-(99–126) [ANF-(99–126)], produced by Mukoyama et al.20 and named MAb KY-ANP-II. MAb KY-ANP-II recognizes human ANF-(99–126) and rat ANF-(99–126) equally and blocks the ability of both exogenous and endogenous ANP to elevate plasma cyclic GMP levels.21 Intraventricular administration of MAb KY-ANP-II has been shown to produce significant reductions in plasma cyclic GMP levels in stroke-prone SHR (SHRSP) and deoxycorticosterone acetate-salt rats, indicating that the antibody can block the activity of rat ANF-(99–126) in the intact rat. We purified IgG containing MAb KY-ANP-II from mouse ascites fluid (1 ml) with a protein A agarose column.22 Retained IgG with MAb KY-ANP-II was eluted from the protein A column with 3 M MgCl and dialyzed against 0.9% saline overnight. We demonstrated that the purified IgG (1.1 mg/ml) fraction containing MAb KY-ANP-II bound 50% of [125I]-ANP (17,000 cpm) at 1:100,000 final dilution in a total volume of 500 μl.23 In addition, we observed that intravenous injection of a 100 μg dose of purified MAb KY-ANP-II inhibited the increase in plasma cyclic GMP induced by administration of exogenous ANP (20 μg/kg i.v.) to the intact rat,24 confirming the previous characterization of Itoh et al.21 The dose of MAb KY-ANP-II (0.55 μg) used in the current experiment is equivalent to the anti-ANP antibody contained in 0.55 μl of mouse ascites fluid. This is 0.5% of the peripheral intravenous dose (100 μl of ascites fluid) of this monoclonal antibody used in previous studies by Itoh et al.21

**Statistical Analysis**

Results are expressed as mean±SEM. The linear portion of the curve relating the change in HR to change in MAP was analyzed by linear regression analysis for each rat during phenylephrine-induced changes in MAP. A mean slope and an average correlation coefficient were calculated for each relation in each strain/treatment group. The slopes of these regression lines, used as an index of baroreceptor reflex sensitivity, were compared by one-way analysis of variance. Significant differences were then subjected to Neuman-Keuls post hoc analysis. Basal levels of MAP and HR were compared using the same statistical methods. A value of *p*<0.05 was considered significant.

**Results**

Thirty SHR-S and 30 WKY rats were studied. Histologic examination confirmed that cannulas were properly placed in C-NTS in 26 SHR-S and 26 WKY rats. In two SHR-S and one WKY rat, the cannula entered the cerebellum; in one SHR-S and two WKY rats, the cannula was placed in the hypoglossal nucleus. In one WKY rat, the cannula penetrated the superior cerebellar vessels; in one SHR-S, the cannula damaged NTS tissue. These four SHR-S and four WKY rats were excluded from the analysis of experimental results. Examination of 1% thionin-stained sections revealed that the extent of spread of the injectate was less than 250 μm (Figure 1). Neurons near the injection tip had normal morphology in Nissl-stained sections, indicating little damage at this site.

SHR-S had significantly higher pretreatment MAP than WKY rats at the time of study (Tables 1 and 2). There was no difference in basal HR among experimental groups (Tables 1 and 2). Body weight was significantly lower in SHR-S than in WKY rats (228.8±1.3 g versus 268.1±1.8; *n*=26 in each group; *p*<0.05).

Phenylephrine-induced increases in MAP were associated with significant reductions in HR in all experi-
TABLE 1. Pretreatment Mean Arterial Pressure and Heart Rate and Linear Regression Values and Correlation Coefficients for the ΔHeart Rate/ΔMean Arterial Pressure Relation During Phenylephrine Infusion Before and After Microinjection of Atrial Natriuretic Peptide Into Caudal Nucleus Tractus Solitarii

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Basal MAP (mm Hg)</th>
<th>Basal HR (bpm)</th>
<th>ΔHR/ΔMAP (bpm/mm Hg)</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR-S</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-ANP</td>
<td>162±5</td>
<td>374±15</td>
<td>-1.03±0.13</td>
<td>0.92±0.02</td>
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<tr>
<td>Post-ANP</td>
<td>156±7</td>
<td>374±10</td>
<td>-0.68±0.04*</td>
<td>0.92±0.01</td>
</tr>
<tr>
<td>Pre-Veh</td>
<td>160±4</td>
<td>396±13</td>
<td>-1.05±0.10</td>
<td>0.96±0.02</td>
</tr>
<tr>
<td>Post-Veh</td>
<td>156±4</td>
<td>393±15</td>
<td>-1.03±0.13</td>
<td>0.92±0.02</td>
</tr>
<tr>
<td>WKY</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-ANP</td>
<td>109±5†</td>
<td>377±16</td>
<td>-2.11±0.30†</td>
<td>0.99±0.01</td>
</tr>
<tr>
<td>Post-ANP</td>
<td>106±4†</td>
<td>366±15</td>
<td>-2.39±0.27†</td>
<td>0.98±0.01</td>
</tr>
<tr>
<td>Pre-Veh</td>
<td>111±3†</td>
<td>380±11</td>
<td>-2.05±0.22†</td>
<td>0.94±0.01</td>
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<tr>
<td>Post-Veh</td>
<td>107±3†</td>
<td>363±11</td>
<td>-2.17±0.41†</td>
<td>0.98±0.01</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; SHR-S, salt-sensitive spontaneously hypertensive rats; ANP, atrial natriuretic peptide; Veh, artificial cerebrospinal fluid vehicle; WKY, Wistar-Kyoto rats. Values are mean±SEM. n=7 in each group.

*p<0.05 compared with the other three SHR-S groups.
†p<0.05 compared with SHR-S groups.

Microinjection of ANP into C-NTS did not alter pretreatment MAP or HR in either SHR-S or WKY rats (Table 1), but did produce a significant reduction in the slope of the ΔHR/ΔMAP relation during phenylephrine infusion in SHR-S (p<0.05) but not in WKY rats (Table 1 and Figure 2). Thus, microinjection of ANP into C-NTS further blunted baroreceptor reflex control of heart rate in the SHR-S. In contrast, microinjection of ACSF into C-NTS did not alter the slope of the ΔHR/ΔMAP relation in either strain (Table 1).

Microinjection of MAb KY-ANP-II into C-NTS increased pretreatment MAP by 15 mm Hg without changing HR in SHR-S (Table 2). There was no change in MAP in WKY rats, as predicted from our previous study. Microinjection of the anti-ANP antibody significantly enhanced the slope of the ΔHR/ΔMAP relation during phenylephrine infusion in SHR-S (p<0.05) but not in WKY rats (Table 2 and Figure 2), indicating that blockade of endogenous ANP in C-NTS improved baroreceptor reflex control of HR in SHR-S but not in WKY rats. In contrast, microinjection of same amount of IgG into NTS did not alter MAP or HR in either strain (Table 2).

Discussion

The principal finding of the current study was that microinjection of a blocking monoclonal antibody to...

TABLE 2. Pretreatment Mean Arterial Pressure and Heart Rate and Linear Regression Values and Correlation Coefficients for the ΔHeart Rate/ΔMean Arterial Pressure Relation During Phenylephrine Infusion Before and After Microinjection of Monoclonal Antibody Into Caudal Nucleus Tractus Solitarii

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Basal MAP (mm Hg)</th>
<th>Basal HR (bpm)</th>
<th>ΔHR/ΔMAP (bpm/mm Hg)</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR-S</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-MAb</td>
<td>152±4</td>
<td>383±5</td>
<td>-0.97±0.12</td>
<td>0.93±0.02</td>
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<tr>
<td>Post-MAb</td>
<td>180±6*</td>
<td>376±9</td>
<td>-1.73±0.30*</td>
<td>0.97±0.01</td>
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<tr>
<td>Pre-IgG</td>
<td>159±3</td>
<td>402±11</td>
<td>-1.09±0.17</td>
<td>0.96±0.01</td>
</tr>
<tr>
<td>Post-IgG</td>
<td>158±5</td>
<td>378±8</td>
<td>-1.02±0.15</td>
<td>0.98±0.01</td>
</tr>
<tr>
<td>WKY</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-MAb</td>
<td>116±3†</td>
<td>376±8</td>
<td>-1.84±0.11†</td>
<td>0.97±0.01</td>
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<tr>
<td>Post-MAb</td>
<td>109±3†</td>
<td>367±9</td>
<td>-1.79±0.18†</td>
<td>0.97±0.01</td>
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<tr>
<td>Pre-IgG</td>
<td>114±4†</td>
<td>376±12</td>
<td>-2.14±0.25†</td>
<td>0.98±0.01</td>
</tr>
<tr>
<td>Post-IgG</td>
<td>106±3†</td>
<td>386±6</td>
<td>-2.07±0.25†</td>
<td>0.97±0.01</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; SHR-S, salt-sensitive spontaneously hypertensive rats; MAb, monoclonal antibody to atrial natriuretic peptide; IgG, immunoglobulin G; WKY, Wistar-Kyoto rats. Values are mean±SEM. n=5–7 in each group.

*p<0.05 compared with the other three SHR-S groups.
†p<0.05 compared with SHR-S groups.
ANP into the caudal NTS of SHR-S enhanced arterial baroreceptor reflex control of HR during phenylephrine-induced increases in MAP. Conversely, microinjection of ANP into the caudal NTS blunted arterial baroreceptor reflex control of HR during phenylephrine infusion in SHR-S. Neither the monoclonal antibody nor ANP had a significant effect on arterial baroreceptor reflex control of HR in WKY rats. Arterial baroreceptor reflex control of HR was impaired in untreated SHR-S compared with WKY rats, as previously reported by our laboratory.25,26 These data suggest that endogenous ANP in the caudal NTS modulates baroreceptor reflex control of HR in the SHR-S but not in WKY rats.

Our finding that microinjection of monoclonal antibody to ANP into the caudal NTS of SHR-S enhanced arterial baroreceptor reflex control of HR during phenylephrine infusion is the first direct demonstration that endogenous ANP in the NTS modulates any aspect of baroreceptor reflex function in this genetic model of hypertension. In contrast, microinjection of the same dose of monoclonal antibody into the caudal NTS of WKY rats had no significant effect on the ΔHR/ΔMAP relation during phenylephrine infusion in salt-sensitive spontaneously hypertensive rats. SHR, spontaneously hypertensive rats; ANP, atrial natriuretic peptide; MAb, monoclonal antibody to atrial natriuretic peptide; HR, heart rate; MAP, mean arterial pressure. *p<0.05 comparison between SHR-S/Post-ANP group and SHR-S/Pre-ANP group; **p<0.05 comparison between SHR-S/Post MAb group and SHR-S/Pre-MAb group.

FIGURE 2. Line graph shows effects of microinjection of atrial natriuretic peptide or monoclonal antibody into caudal nucleus tractus solitarii on the slope of the ΔHR/ΔMAP relation during phenylephrine infusion in salt-sensitive spontaneously hypertensive rats. SHR, spontaneously hypertensive rats; ANP, atrial natriuretic peptide; MAb, monoclonal antibody to atrial natriuretic peptide; HR, heart rate; MAP, mean arterial pressure. *p<0.05 comparison between SHR-S/Post-ANP group and SHR-S/Pre-ANP group; **p<0.05 comparison between SHR-S/Post MAb group and SHR-S/Pre-MAb group.

blood pressure that followed antibody injection as the consequence of removing endogenous ANP, which we hypothesize causes tonic activation of NTS neurons in SHR-S. Tonic activation of NTS neurons by ANP would tend to buffer the hypertension in SHR-S, so that the hypertension would become more severe when the ANP was removed after administration of the antibody. The absence of a pressor response to administration of the antibody into the NTS of WKY rats suggests that NTS neurons are not tonically activated by ANP in the normotensive WKY.

Studies from a number of laboratories have shown that both arterial and cardiopulmonary components of the baroreceptor reflex are impaired in SHR.25–30 Elevation in either arterial pressure or right atrial pressure produces significantly less inhibition of sympathetic nerve activity in SHR than in normotensive controls.28,29 Further, the defect in arterial baroreceptor reflex control in SHR has been localized to the central nervous system, since stimulation of the aortic depressor nerve in decerebrate or urethane-anesthetized SHR produces a significantly smaller depressor effect and significantly less suppression of splanchnic nerve activity in SHR than in WKY rats.30 Our own laboratory has demonstrated that both arterial and cardiopulmonary baroreceptor reflex–mediated control of lumbar sympathetic nerve activity and HR are blunted in SHR-S compared with SHR-R and normotensive WKY and Sprague-Dawley rats consuming a basal (1%) NaCl diet.25–27 Tonic activation of the central baroreceptor reflex arc by ANP in the SHR-S could blunt baroreceptor reflex responsiveness to stimulation by volume expansion and phenylephrine infusion in SHR-S, contributing to the central defect in the baroreceptor reflex pathway previously described in SHR.30 Our findings that arterial baroreceptor reflex control of HR was sensitized in response to reductions in local (caudal NTS) ANP concentration by microinjection of a blocking antibody and was blunted in response to enhancement in local ANP concentration by microinjection of exogenous ANP are consistent with this hypothesis.

References

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