Natriuretic peptide receptor A mediates renal sodium excretory responses to blood volume expansion

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IN RESPONSE TO AN increase in atrial distension, the peptide hormone atrial natriuretic peptide (ANP) is released into the circulation and elicits natriuresis, diuresis, and vasodilation (8, 25). Acting on natriuretic peptide receptors, ANP inhibits salt and water reabsorption in proximal tubule and inner medullary collecting duct cells and inhibits renin and vasopressin release as well as aldosterone synthesis and secretion (1, 4, 7, 47).

Natriuretic peptides belong to a family of three homologous peptide hormones. ANP and brain natriuretic peptide (BNP) are released by the heart; and C-type natriuretic peptide (CNP) is produced in endothelial cells (10, 20, 46). All three natriuretic peptides are thought to exert important roles in the maintenance of blood pressure and cardiovascular homeostasis. Distinct natriuretic peptide receptors have been identified and characterized by molecular cloning (39, 41). These include natriuretic peptide receptor A, B, and C, also designated as NPRA, NPRB, and NPRC, respectively (11, 19, 33). Both ANP and BNP specifically bind to NPRA, whereas CNP binds to NPRB; nevertheless, all three natriuretic peptides show affinity to NPRC. The hormone binding to NPRA and NPRB results in the production of intracellular second messenger cGMP by guanylyl cyclase activity that resides in the intracellular domains of these receptors (22, 36, 43). NPRA is thought to be the primary ANP/BNP signaling molecule and has been suggested as the principal mediator of natriuretic peptide activities.

Previous experimental data established that ANP plays an important role in regulation of renal function by its vasodilatory and natriuretic responses and its ability to counteract the renin-angiotensin-aldosterone system in a tissue-specific manner (25). Attempts have been made to define physiological responses of ANP using several experimental approaches. It has been possible to correlate the effects of changes in blood hormone levels commensurate with those found in pathophysiological states by infusing the exogenous hormones (40). Cardiac appendectomy has been used to prevent ANP release; however, the problem in this setting is that the missing normal cardiac function results in a lack of physiological reflexes that are normally elicited by atria (42). Other studies used monoclonal antibodies against circulating ANP and agents that specifically inhibit the signaling pathway of NPRA by blocking cGMP production. Although two com-
pounds, A-71915 and HS-142–1, have been shown to diminish the effect of ANP by antagonizing NPRA; these compounds do not completely inhibit NPRA and may have nonspecific effects (9, 30). Gene-targeting strategies in mice provide novel approaches in the study of the physiological responses corresponding to gene dosage in vivo (15, 45).

Genetic mouse models with disruption of the ANP/NPRA system have provided strong support for a physiological role of this hormone-receptor system in the regulation of arterial pressure and other pathophysiological functions (13, 14, 21, 24, 31, 37, 44). Therefore, the genetic defects that reduce the activity of ANP and its receptor system can be considered as candidate contributors to essential hypertension and congestive heart failure (12, 14, 17, 18, 31, 44, 48). To examine the regulatory role of NPRA in kidney function and blood pressure homeostasis at the molecular level, we performed studies evaluating the changes in renal function using Npr1 (coding for NPRA) gene-disrupted and gene-duplicated mutant mouse models. We hypothesized that the quantitative genetic alterations in NPRA expression levels in vivo mediate the primary contributors to essential hypertension and congestive heart failure (12, 14, 17, 18, 31, 44, 48). To test this hypothesis, we administered uretic and hemodynamic responses to intravascular expansion not accompanied by hemodilution.

MATERIALS AND METHODS

Generation of mice and genotyping. Npr1 gene-disrupted and gene-duplicated mice were generated by homologous recombination in embryonic stem cells as previously described (31, 32). Animals were bred and maintained at the Animal Care Facility of Tulane University Health Sciences Center and handled under protocols approved by the Institutional Animal Care and Use Committee. Npr1 genotypes used in the present studies were littermate progenies of a mixed 129/C57BL6 genetic background and have been designated as follows: homozygous mutant allele (+/−; 0-copy), wild-type allele (+/+; 2-copy), and gene-duplicated allele (++/+; 4-copy). The breeding of 1-copy (+/−) heterozygous animals generated progenies consisting of 0-, 1-, and 2-copy mice. These animals were genotyped by multiple PCR analysis of DNA isolated from tail biopsies using primer A (5′-GCT CTC TTG TCG CCG AAT CT-3′), corresponding to a sequence 5′ to the mouse Npr1 gene common to both alleles (2-copy); primer B (5′-TGT CAC CAT GGT CTG ATC GC-3′), corresponding to an exon 1 sequence only present in the intact mouse allele (1-copy); and primer C (5′-GCT TCC TCG TGG TTT AGC GT-3′), a sequence in the neomycin resistance cassette only present in the null allele (0-copy). The PCR reaction from tail DNA included 50 mM Tris-HCl (pH 8.5), 20 mM ammonium sulfate, 1.5 mM MgCl2, 10% DMSO, 100 μM each of dNTPs, 2 U of Taq DNA polymerase, and 40 nM primers. The PCR for 0-, 1-, and 2-copy mice was performed by the use of a 60-s denaturation step at 94°C, a 60-s annealing step at 60°C, and a 60-s extension step at 72°C, respectively, for 35 cycles using DNA Thermal Cycler 480 as previously described (44) with modifications. PCR products were resolved on 2% agarose gels with the endogenous band of 500 bp and targeted band of 200 bp (Fig. 1A).

The breeding of gene-duplicated 3-copy (+/+ +/+ +) heterozygous mice generated progenies consisting of 2-, 3-, and 4-copy animals. The Npr1 gene-duplicated mice were genotyped using upstream (5′-CCT CTA GAT GCA TAC ATG TCG C-3′) and downstream (5′-CTG CAA GTT AAG TGT ATT TTT TTC-3′) primers. The PCR reaction from tail DNA included 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 4 mM MgCl2, 0.4 mM each of dNTPs, 2.5 U of Taq DNA polymerase, and 40 nM primers. The PCR was performed by the use of a 30-s denaturation step at 94°C, a 30-s annealing step at 60°C, and a 30-s extension step at 72°C, respectively, for 35 cycles as previously described (32) with modifications. The amplified genomic fragments were separated on nondenaturing acrylamide gels and corresponded to 108- and 124-bp bands from 2- and 4-copy genotypes, respectively, whereas the heterozygous (3-copy) animals contained both 108- and 124-bp amplified genomic fragments (Fig. 1B).

Animal preparation. The mice were housed under a 12:12-h light-dark cycle at 25°C and fed regular chow (Purina Laboratory) and tap water ad libitum. In the present experiments, 16- to 24-wk-old male mice weighing 30–35 g were used. Animals were anesthetized with Inactin (100 mg/kg ip thiobutabarbital sodium). A supplemental dosage of anesthetic (5 mg/kg im ketamine) was administered as required. The animals were placed on a servo-controlled surgi-
cal table that maintained body temperature at 37°C, and a tracheotomy was performed. The animals were allowed to breathe humidified 95% O₂-5% CO₂ by placing the exterior end of the tracheal cannula inside a small plastic chamber. The right jugular vein was catheterized with PE-10 tubing for fluid infusion. After catheterization, 0.9% NaCl containing 10% Inutest (Laevasom-Gesellschaft, Lint, Austria), 3% PAH (Sigma), and 1% BSA was infused at a rate of 2.5 µl/min. The left carotid artery was cannulated (PE-10 tubing connected to PE-50 tubing) for measurements of arterial pressure. Blood pressures were recorded on a pressure transducer connected to a Grass polygraph (Grass Instrument, Quincy, MA). Blood pressures were determined continuously throughout the duration of the experiment. The bladder was catheterized with PE-50 tubing via a supra pubic incision for urinary collections as previously described (6). After equilibration for 45 min, eight consecutive 20-min urinary collections were obtained. At the end of the experiment, blood was collected from the carotid artery into hemocrit tubes and chilled tubes containing 5 µl of 0.2 M sodium-EDTA and was immediately centrifuged at 4°C. Plasma was removed and stored at -80°C until used for analysis. Plasma and urine were analyzed for protein content, Inutest, and PAH concentrations.

Volume expansion. Npr1 homozygous mutant (0-copy; n = 9), wild-type (2-copy; n = 9), and gene-duplicated (4-copy; n = 9) mice received whole blood obtained from age-matched 1-copy (n = 18) or 3-copy (n = 9) male donor mice. The donor animals were anesthetized with Inactin (100 mg/kg ip), and the whole blood was drawn by cardiac puncture using a heparinized syringe. Hemodynamic and kidney functions were determined before (0–60 min), during (60–80 min), and after (80–160 min) blood volume expansion. Before the volume expansion period (0–60 min), three initial consecutive 20-min urine collections and blood pressure measurements were performed, after which the blood volume expansion was carried out beginning at 60 min. Fresh whole blood was infused into 0-, 2-, and 4-copy mice over a 20-min volume expansion period (60–80 min) to expand the circulating blood volume by an estimated 15% (450 µl, ~1.5% of body wt). Five consecutive 20-min recovery (60–160 min) blood pressure measurements and urinary collections were performed during and after the blood infusion. One hundred microliters of blood were collected at 60 and 100 min, and total blood was collected at 160 min after the completion of the experiment.

Determination of plasma and urinary inulin (Inutest) and PAH. Ten microliters of plasma were mixed with 90 µl of 0.05 M sodium acetate buffer, pH 6.2, and cGMP in the samples was determined with a radioimmunoassay kit (Peninsula, Belmont, CA) as previously described (35).

RESULTS

Urinary cGMP and plasma ANP concentrations. As shown in Fig. 2, before volume expansion, urinary cGMP excretion rates (pmol·min⁻¹·g kidney⁻¹) were significantly lower in 0-copy mice (3.5 ± 0.9; P < 0.05) and significantly higher in 4-copy mice (14.7 ± 1.3; P < 0.05) compared with 2-copy wild-type mice (7.6 ± 1.0). Interestingly, during volume expansion, urinary cGMP excretion rates were dramatically increased in both 2- and 4-copy mice; however, the cGMP values were significantly higher in 4-copy mice (27.8 ± 2.1; P < 0.01) compared with 2-copy wild-type animals (14.2 ± 1.9). On the other hand, the urinary cGMP excretion rate was significantly reduced in 0-copy mice (1.0 ± 0.05; P < 0.01). After volume expansion, the urinary cGMP excretion rates were 0.7 ± 0.1, 3.6 ± 1.6, and 10.5 ± 2.0 in 0-, 2-, and 4-copy mice, respectively.

Figure 3 shows that after volume expansion, the plasma ANP concentrations (pmol/ml) were significantly higher in 0-copy mice (2.5 ± 0.2; P < 0.01) compared with 2- and 4-copy mice.
compared with 2-copy (1.5 ± 0.2) and 4-copy (0.7 ± 0.1) mice, respectively.

Plasma protein concentrations and hematocrits. The plasma protein concentrations were not significantly different among either donor (1- and 3-copy) or recipient (0-, 2-, and 4-copy) mice (Fig. 4A). Similarly, there were no significant differences in hematocrits in the donor mice. However, after volume expansion, the hematocrits in recipient 2- and 4-copy mice were significantly higher compared with recipient 0-copy mice (Fig. 4B).

Mean arterial blood pressures before, during, and after whole blood volume expansion. The mean arterial pressures during all conditions were 30–40 mmHg higher in Npr1 homozygous null mutant (0-copy) mice than in wild-type (2-copy) mice (Fig. 5). In contrast, the mean arterial pressures were 15–20 mmHg lower in Npr1 gene-duplicated (4-copy) mice than in wild-type (2-copy) control animals. Before volume expansion (0–60 min), the mean arterial pressures (mmHg) were significantly higher in 0-copy mice (129 ± 4; *P < 0.01) and significantly lower in 4-copy mice (77 ± 2; P < 0.01) compared with 2-copy wild-type mice (92 ± 3). During volume expansion (60–80 min), the mean arterial pressures increased and remained at significantly higher levels in 0-copy mice (140 ± 4; *P < 0.001) but remained at significantly lower levels in 4-copy mice (90 ± 3; P < 0.01) compared with 2-copy wild-type mice (108 ± 3). Even after volume expansion (80–160 min), the mean arterial pressures remained at significantly higher levels in 0-copy mice (136 ± 4; *P < 0.001) and at significantly lower levels in 4-copy mice (81 ± 3; *P < 0.01) compared with 2-copy wild-type animals (95 ± 4).

GFR and RPF. As shown in Fig. 6A, before volume expansion, the baseline (0–60 min) GFR (ml·min⁻¹·g kidney wt⁻¹) was significantly lower in 0-copy mice (0.49 ± 0.03; *P < 0.05) and significantly higher in 4-copy mice (0.81 ± 0.04; *P < 0.05) compared with 2-copy wild-type counterparts (0.63 ± 0.03). During the
volume expansion period, GFR increased in all groups, which was maintained at a significantly lower level in 0-copy mice (0.58 ± 0.04; P < 0.01) and at a significantly higher level in 4-copy mice (1.19 ± 0.12; P < 0.01) compared with 2-copy wild-type animals (0.82 ± 0.09). Nevertheless, after volume expansion, GFR still remained at a significantly lower level in 0-copy mice (0.49 ± 0.05; P < 0.05) and at a significantly higher level in 4-copy mice (0.87 ± 0.08; P < 0.05) compared with 2-copy wild-type counterparts (0.68 ± 0.06).

Similarly, as shown in Fig. 6B, the baseline RPF (ml·min⁻¹·g kidney wt⁻¹) was significantly lower in 0-copy mice (2.24 ± 0.41; P < 0.05) and significantly higher in 4-copy mice (4.00 ± 0.49; P < 0.05) compared with 2-copy wild-type animals (2.96 ± 0.17). During volume expansion, the RPF remained at a significantly lower level in 0-copy mice (2.72 ± 0.37; P < 0.01) and increased significantly in 4-copy mice (5.78 ± 0.50; P < 0.01) compared with 2-copy wild-type control mice (4.36 ± 0.41). Again, after volume expansion, the RPF was lower in 0-copy mice (2.29 ± 0.30; P < 0.05) and remained significantly elevated in 4-copy mice (4.48 ± 0.47; P < 0.05) compared with 2-copy wild-type animals (3.10 ± 0.34).

**Urinary flow and urinary sodium and potassium excretion.** During volume expansion, urinary flow (µl·min⁻¹·g kidney wt⁻¹) in 2-copy animals increased from 4.9 ± 1.0 to 14.4 ± 1.8 and sodium excretion rate (µeq·min⁻¹·g kidney wt⁻¹) from 1.15 ± 0.22 to 3.11 ± 0.60 (Fig. 7, A and B). In contrast, 0-copy animals exhibited only a small change in urinary flow (3.4 ± 0.2 to 5.0 ± 1.0; P < 0.001) and sodium excretion (0.69 ± 0.21 to 1.10 ± 0.18; P < 0.001) despite the greater increases in arterial pressures. Interestingly, 4-copy mice showed significantly higher urinary flow (6.5 ± 0.6 to 24.0 ± 2.5; P < 0.01) and sodium excretion (1.86 ± 0.23 to 4.75 ± 0.60; P < 0.01) compared with 2-copy control animals (Fig. 7, A and B). Even after volume expansion, both 2- and 4-copy mice had significantly higher urinary flows and sodium excretion rates compared with 0-copy mice. In contrast, urinary potassium excretion rates were not significantly different in any of the Npr1 genotypes before, during, and after volume expansion and were not increased by blood volume expansion (Fig. 7C).

As shown in Fig. 8, before volume expansion, the percent baseline fractional sodium excretions remained at a significantly lower level in 0-copy mice (0.76 ± 0.14%) and were not significantly different in 4-copy (1.51 ± 0.14%) mice compared with 2-copy (1.32 ± 0.13%) wild-type mice. However, during volume expansion, fractional sodium excretions were significantly elevated in 4-copy mice (3.65 ± 0.22%; P < 0.01) and remained significantly lower in 0-copy mice (1.23 ± 0.16%; P < 0.01) compared with 2-copy wild-type animals (2.45 ± 0.16%). After volume expansion, fractional sodium excretions were still significantly higher in 2-copy (1.64 ± 0.16%) and 4-copy (1.90 ± 0.18%) mice compared with 0-copy mice (1.14 ± 0.15%). No significant differences in fractional potassium excretions were observed among the three groups of Npr1 mice (data not shown).

**DISCUSSION**

The present study examined the role of NPRA in the renal and arterial pressure responses to blood volume expansion in Npr1 homozygous null mutant (0-copy), wild-type (2-copy), and gene-duplicated (4-copy) mice. The Npr1 gene-disrupted (0-copy) mice showed the

**Fig. 6.** Effect of volume expansion (VE) on glomerular filtration rate (GFR; A) and renal plasma flow (RPF; B) in Npr1 null mutant (0-copy; n = 9), wild-type (2-copy; n = 9), and gene-duplicated (4-copy; n = 9) mice. Both GFR and RPF were measured in 3 consecutive periods at before (0–60 min), during (60–80 min), and after (80–160 min) pure blood VE. *P < 0.05; **P < 0.01.
highest blood pressures, whereas the Npr1 gene-duplicated (4-copy) mice had the lowest blood pressures compared with wild-type (2-copy) mice. During the period of volume expansion with whole blood infusion, the mean arterial blood pressures increased in all three genotypes; however, the mean arterial pressures were always significantly lower in 4-copy mice and significantly higher in 0-copy mice compared with 2-copy animals. Because of the slow infusion rate, the changes in arterial pressure in response to blood volume expansion were modest, although they appeared to be slightly greater in 2- and 4-copy mice. The renal hemodynamic function was different in the three groups. GFR was 25 to 35% lower in 0-copy mice and 30 to 45% higher in gene-duplicated (4-copy) mice compared with 2-copy control animals. It is noteworthy that 4-copy mice had higher GFR and RPF values during the volume expansion period, whereas 0-copy mice showed significantly lower GFR or RPF responses to the volume expansion. Furthermore, during the volume expansion, 2-copy wild-type animals exhibited significantly higher urinary flow and sodium excretory responses compared with 0-copy null mutant mice despite the greater mean arterial pressures in the 0-copy mice. Interestingly, 4-copy mice showed even greater urinary flow and sodium excretory responses than 2-copy mice. Urinary potassium excretion rates were not significantly different among three Npr1 genotypes before, during, or after volume expansion.

A number of factors influence the kidney’s ability to excrete sodium and water in response to blood volume expansion (2, 3, 23, 27, 28). Activation of natriuretic systems such as natriuretic peptides (ANP, BNP) and nitric oxide enhances the pressure-natriuresis relationship and reduces arterial pressures. It has also been suggested that chloride-mediated feedback control of NPRA occurs in the kidney and probably plays a role in the regulation of ANP-mediated natriuresis (26). Initial studies have shown that ANP suppresses renin and decreases blood pressures (5, 24, 29). Our earlier findings with Npr1 gene-disrupted mice demonstrated that, at birth, the absence of NPRA allows greater renin and ANG II levels and increased renin mRNA expression compared with 2-copy control mice (44). However, at 3–16 wk of age, both circulating and kidney renin and ANG II levels were decreased dramatically in 0-copy mice compared with 2-copy control animals. This decrease in the renin activity in adult 0-copy mice could be due to progressive elevation in arterial pressures leading to inhibition of renin synthesis and release from the kidney juxtaglomerular cells. However, aldosterone levels in 0-copy mice were dissociated from the circulating ANG II levels and were elevated. It is also possible that the renin in 0-copy mice might be suppressed as a consequence of aldosterone-mediated sodium retention with associated fluid retention (44). These and other various systems could be responsible for the differences in basal values among the three groups.

Previous studies suggested that increased levels of ANP released into the plasma in response to blood volume expansion in rats were mainly responsible for the natriuretic and diuretic responses (3, 4, 40).
thermore, Paul et al. (40) showed that both ANP and acute blood volume expansion act on the kidney through a similar saturable mechanism. However, direct evidence demonstrating the quantitative contribution of ANP working through NPRA to the natriuresis and diuresis resulting from an isohemic, isoosmotic blood volume expansion has not been obtained. In a study using Npr1 homozygous null mutant and wild-type mice, it was demonstrated that infusion of ANP, while causing substantial natriuresis and diuresis in wild-type mice, did not cause significant increases in sodium excretion or urinary flow in NPRA-deficient mice (16). Furthermore, urinary flow and sodium excretion rapidly increased in response to volume expansion with albumin containing Ringer solution in wild-type (2-copy) mice compared with homozygous null mutant (0-copy) mice. In the present study, we examined the quantitative contribution and possible mechanisms mediating the responses of NPRA by determining the RPF, GFR, urinary flow, and sodium and potassium excretion patterns following blood volume expansion in 0-, 2-, and 4-copy mice in a Npr1 gene dose-dependent manner. By using whole blood, hemodilution did not occur and plasma protein levels were not reduced. Thus other natriuretic mechanisms related to plasma dilution, such as decreases in colloid osmotic pressure or decreases in hematocrit, were not activated with this protocol. Although the blood volume expansion stimulated the release of ANP in all three Npr1 genotypes of mice, significant functional responses occurred only in 2- and 4-copy mice but not in 0-copy animals. These results demonstrate that the ANP/NPRA axis is primarily responsible for mediating the renal hemodynamic and sodium excretion responses to intravascular blood volume expansion. Furthermore, the sodium excretion responses appear to be due to the combined contribution of increases in filtered load as well as reductions in tubular fractional reabsorption. The associated changes in urinary cGMP excretion rates are consistent with the activation of tubular NPRA leading to increased formation of cGMP, which might pass into tubular fluid and be excreted in the urine. In addition, it should be noted that the increase in urinary flow could be due, in part, to an inhibition of antidiuretic hormone levels via the low-pressure volume receptors stimulated by intravascular volume expansion.

The finding that the absence of NPRA almost completely prevented the sodium excretory responses to blood volume expansion is somewhat surprising in that there are multiple systems that respond to volume expansion. An important part of the experimental design was to minimize nonspecific responses that could be associated with hemodilution, reductions in plasma colloid osmotic pressure, and other compositional changes in the blood that could directly affect sodium reabsorption and/or GFR. In addition, the blood volume infusion period was extended over a 20-min period to minimize reflexogenic alterations in sympathetic tone and volume expansion-mediated inhibition of vasopressin release. In this setting, it was possible to demonstrate the critical role that NPRA exerts in mediating the sodium excretory responses to a selective stimulus associated with blood volume expansion, which is presumably due to increases in right atrial pressure caused by the blood infusion.

Earlier studies suggested that the ANP/NPRA system plays an important role in blood pressure homeostasis by direct natriuretic, diuretic, and vasodilatory actions on the kidneys (4, 25). ANP-deficient genetic strains of mice demonstrated that a defect in ANP synthesis can cause hypertension in homozygous null mutant mice with no circulating or cardiac ANP (14). Therefore, genetic defects that reduce the activity of the natriuretic peptide system can be considered as candidate contributors to essential hypertension. Mice lacking the ANP gene function and kept on a high-salt diet (8% NaCl) showed hypertension with increased arterial pressures of ~22 mmHg, which suggested that genetically reduced production of ANP can lead to salt-sensitive hypertension (14). Npr1 gene-deficient mice used in the present study exhibited a higher mean arterial pressure on high-salt diet compared with animals kept on medium- or low-salt diets (32). The absence of NPRA expression in 0-copy mice provoked salt-sensitive increases in blood pressures, whereas an increased expression of NPRA in 4-copy mice was able to lower the blood pressures and protected against high dietary salt intake. These previous studies provide evidence that ANP can be considered a major system contributing an important role in the regulation of blood volume and altered blood pressure. However, it should be acknowledged that Lopez et al. (21) failed to show salt-sensitive hypertension in their Npr1 gene-deficient mice. Thus it is likely that ANP/NPRA axis serves an important mediator in acute natriuresis and diuresis after blood volume expansion.

In conclusion, our present results demonstrate that both GFR and RPF were significantly lower in 0-copy and higher in 4-copy mice compared with 2-copy wild-type animals before, during, and after volume expansion. The data show that ANP responses to volume expansion led to the significantly lesser excretion of sodium and water in 0-copy mice and significantly greater excretory responses along with reduced tubular reabsorption in 4-copy mice compared with 2-copy (wild type) mice. Similarly, during the volume expansion, urinary cGMP concentration was significantly lower in 0-copy mice and greater in 4-copy mice compared with 2-copy control animals. Thus the higher cGMP concentrations in urinary samples were increased corresponding to Npr1 gene copy numbers. Our findings establish that NPRA is critical in mediating the natriuresis, diuresis, and renal hemodynamic responses to acute blood volume expansion.
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DISCLOSURES

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REFERENCES


