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α2δ-1–Dependent NMDA Receptor Activity in the Hypothalamus Is an Effector of Genetic-Environment Interactions That Drive Persistent Hypertension

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The interplay between genetic and environmental factors is critically involved in hypertension development. The paraventricular nucleus (PVN) of the hypothalamus regulates sympathetic output during stress responses and chronic hypertension. In this study, we determined mechanisms of synaptic plasticity in the PVN in chronic stress-induced persistent hypertension in male borderline hypertensive rats (BHR), the first offspring of spontaneously hypertensive rats and normotensive Wistar-Kyoto rats. In Wistar-Kyoto rats, chronic unpredictable mild stress (CUMS) increased arterial blood pressure (ABP) and heart rate, which quickly returned to baseline after CUMS ended. In contrast, in BHR, CUMS caused persistent elevation in ABP, which lasted at least 2 weeks after CUMS ended. CUMS also increased the mRNA level of α2δ-1 and synaptic protein levels of GluN1, α2δ-1, and α2δ-1–GluN1 complexes in the PVN in BHR. Furthermore, CUMS significantly increased the frequency of miniature EPSCs and the amplitude of NMDAR currents in spinally projecting PVN neurons in BHR; these increases were normalized by blocking NMDARs with AP5, inhibiting α2δ-1 with gabapentin, or disrupting the α2δ-1–NMDAR interaction with α2δ-1Tat peptide. Microinjection of AP5 or α2δ-1Tat peptide into the PVN normalized elevated ABP and renal sympathetic nerve activity in stressed BHR. In addition, systemically administered gabapentin or memantine attenuated higher ABP induced by CUMS in BHR. Our findings indicate that chronic stress-induced persistent hypertension is mediated by augmented synaptic outflow via α2δ-1–bound NMDARs in the PVN. This new information provides a cellular and molecular basis for how the genetic-environment interactions cause persistent hypertension.

Key words: autonomic nervous system; depression; gabapentinoids; neurogenic hypertension; stress; sympathetic nervous system

Significance Statement

Chronic stress is a major risk factor for hypertension development, especially for individuals with a genetic predisposition to hypertension. Using a rat model of borderline hypertension, we showed that chronic stress induced long-lasting hypertension and sympathetic nerve hyperactivity, which were maintained by NMDAR activation in the hypothalamus. Chronic stress also increased the expression of α2δ-1, previously regarded as a Ca2+ channel subunit, promoting physical interaction with and synaptic trafficking of NMDARs in the hypothalamus. Inhibiting α2δ-1, blocking NMDARs, or disrupting α2δ-1–bound NMDARs reversed chronic stress-induced sympathetic outflow and persistent hypertension. Thus, α2δ-1–dependent NMDAR activity in the hypothalamus is an effector of genetic-environment interactions and may be targeted for treating stress-induced neurogenic hypertension.

Introduction

Essential hypertension often results from complex interactions between genetic and environment factors and is a major risk factor for stroke, renal failure, and ischemic heart disease. Individuals with a genetic predisposition to hypertension are prone to developing hypertension during their adulthood (Widgren et al., 1992; Wang et al., 2008). Stressful situations typically cause a temporary elevation of arterial blood pressure (ABP), while untreated chronic stress often leads to the development of sustained hypertension, particularly in individuals with genetic traits increasing their risk of hypertension. Borderline
hypertensive rats (BHR), the first offspring of spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY), are a commonly used genetic model for studying the genetic-environment interaction in the development of hypertension (Lawler et al., 1988; Sanders and Lawler, 1992; Sarenac et al., 2011). Adult BHR have higher baseline ABP compared with normotensive controls and exhibit higher ABP when exposed to stress or a high-salt diet (Lawler et al., 1988). However, the cellular and molecular mechanisms involved in the genetic-environment interaction leading to chronic hypertension in BHR remain poorly understood.

The paraventricular nucleus (PVN) of the hypothalamus is a critical brain region integrating endocrine and autonomic responses to stress (Swanson and Sawchenko, 1980; Ulrich-Lai and Herman, 2009). The PVN is also a primary locus generating excess sympathetic outflow in hypertension through its projection to sympathetically related sites in the brainstem and spinal cord (Strack et al., 1989; Pyner and Coote, 1999; Dampney et al., 2018). Overactivation of the sympathetic nervous system is a predominant mechanism of stress-induced hypertension (Alkadhi et al., 2005; Sarenac et al., 2011). Although the sympathetic output generated in the PVN is crucially involved in the pathogenesis of hypertension in SHR (Allen, 2002; Li and Pan, 2007), it is unclear how chronic stress impacts neuronal and synaptic plasticity in the PVN to control sympathetic drive in hypertension.

The glutamate NMDAR activity in the PVN plays a key role in elevated sympathetic output in SHR (Li and Pan, 2007; Li et al., 2008). NMDARs are now known to interact with α2δ-1, the binding protein of gabapentinoids (Gee et al., 1996; Fuller-Bicer et al., 2009), which are clinically used to treat epilepsy, anxiety, and neuropathic pain. Recent studies revealed that α2δ-1 can physically interact with NMDARs to promote synaptic trafficking of NMDARs independent of the conventional role of α2δ-1 as a voltage-gated calcium channel subunit (J. Chen et al., 2018; Ma et al., 2018a). Also, α2δ-1 is required for the increased synaptic NMDAR activity in the PVN, which maintains elevated sympathetic nerve discharges in SHR (Ma et al., 2018b). However, it is not known whether NMDARs and α2δ-1 in the PVN play a role in chronic stress–induced sympathetic output and hypertension.

Chronic unpredictable mild stress (CUMS) is a widely used and effective paradigm to induce depressive- and anxiety-like behaviors in rodents (Haile et al., 2001; Lu et al., 2019). In this study, we determined the synaptic mechanism underlying CUMS-induced hypertension development in BHR. We found that the ABP in CUMS-treated BHR reached the same level as in SHR and remained elevated for at least 2 weeks after discontinuance of CUMS. Importantly, this CUMS-induced persistent hypertension in BHR was maintained by increased sympathetic drive via α2δ-1–dependent synaptic NMDAR activity in the PVN. This new information suggests that α2δ-1–coupled NMDARs could be targeted to treat stress-induced sympathetic outflow and hypertension.

Materials and Methods
Animal models and CUMS. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Texas MD Anderson Cancer Center and conformed to the National Institutes of Health guidelines on the ethical use of animals. Male WKY (10 weeks old) and female SHR (10 weeks old) were purchased from Harlan Laboratories (Envigo). BHR were the first offspring obtained by crossing male WKY with female SHR. ABP was initially measured by using a tail-cuff method (Table 1), and 10-week-old male BHR and WKY were used in final experiments. All animals were housed (3 rats per cage) at the animal facility on a 12 h light/dark cycle, with free access to food and water (with the exception of the stressors described below).

CUMS was elicited as described previously (Haile et al., 2001; Zhou et al., 2018). In brief, BHR and WKY were exposed to 2 random stressors per day from a total of 8 possible stressors for 42 d. The stressors include cage rotation, cold isolation at 4°C, light off, light on, forced swim, restraint stress, isolation housing, and food/water deprivation (Table 2). The unstressed control BHR and WKY were housed in their home cages during the same period.

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*Oversight is from 7:00 P.M. to 7:00 A.M. on the next day.
ABP in freely moving, conscious rats was measured by using implanted telemetry (Telemetry Research) connected to a Millar catheter. Briefly, rats were anesthetized with 2% isoflurane, and the catheter connected to the telemetry transmitter was inserted into the descending aorta (Ye et al., 2011; Qiao et al., 2017). The transmitter was implanted and fixed in the abdominal cavity with a suture to the peritoneum. The abdominal wound was closed in 2 layers with interrupted sutures. Rats were then given buprenorphine (0.05 mg/kg, s.c., every 12 h for 2 d) and enrofloxacin (5 mg/kg, s.c., daily for 3 d) and housed singly for recovery for 7 d. ABP signals were continuously recorded and analyzed using LabChart 7 (AD Instruments), and heart rate (HR) values were derived from the ABP pulse signal.

**Synaptosomes and coimmunoprecipitation.** Rats were anesthetized with 3% isoflurane and then rapidly decapitated. The brain was quickly removed and placed in ice-cold aCSF saturated with 95% O2 and 5% CO2. The PVN tissues were obtained using a punch micro-dissection technique (Ye et al., 2011; Ma et al., 2018b). To isolate synaptosomes, we homogenized PVN tissues (pooled from 3 rats per sample) using 10 volumes of ice-cold HEPES-buffered sucrose solution (0.32 mol/L sucrose, 1 mmol/L EGTA, and 4 mmol/L HEPES at pH 7.4) containing a protease inhibitor cocktail. The homogenates were then centrifuged at 2000 × g for 10 min at 4°C to remove nuclei and large debris, and the supernatant was collected. The homogenates were then centrifuged at 12,000 × g for 10 min at 4°C to obtain the synaptosomal fraction (Ma et al., 2018b).

The synaptosomal samples were incubated at 4°C overnight with Protein G beads (#16-26-26; EMD Millipore) prebound to a rabbit anti-GluN1 antibody (#G8913; dilution 1:100; Sigma-Aldrich). Protein G beads prebound to rabbit IgG were used as controls. Protein G beads were rotated at 4°C overnight and then washed 3 times with immunoprecipitation buffer. Protein samples on the beads were isolated using loading sample buffer and were separated by SDS-PAGE. The membranes were blotted with 5% nonfat dry milk and incubated with a mouse anti–α2δ-1 antibody (#sc-271697; dilution 1:500; Santa Cruz Biotechnology) at 4°C overnight. The blotting membranes were incubated with an HRP-conjugated anti-mouse antibody and then subjected to a PVDF membrane.

**Samples and cDNA preparation.** Total RNA was isolated from the PVN using a modified TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. cDNA (1 μg) was reverse-transcribed to cDNA using SuperScript III (Invitrogen). The amplification reaction system was composed of a mixture of 12.5 μL of SYBR Green PCR master mix (Bio-Rad), 1 μL of cDNA, and a 200 nmol/L primer in a total volume of 20 μL. The amplification cycling conditions were as follows: 2 min at 50°C, 10 min at 95°C, and 40 cycles at 95°C for 15 s, followed by 1 min at 60°C. The PCR products were stained with ethidium bromide and visualized under UV interference contrast optics.

**Hypothalamic slices.** Coronal hypothalamic slices (300 μm thick) were cut and incubated in aCSF continuously gassed with a mixture of 95% O2 and 5% CO2 at 34°C for 1 h before electrophysiological recordings. The tissue slices were placed in a recording chamber filled with aCSF (pH adjusted to 7.2–7.4 with 1 m KOH; 290–300 mOsm/L). The recording electrode was 3-6 MΩ when filled with an internal solution containing (in mmol/L) as follows: 135.0 potassium glutonate, 5.0 tetraethylammonium, 2.0 MgCl2, 0.5 CaCl2, 5.0 HEPES, 5.0 EGTA, 5.0 Mg-ATP, 0.5 Na2GTP, and 10 lidocaine N-ethyl bromide (pH adjusted to 7.2–7.4 with 1 m KOH; 290–300 mOsm/L). To record evoked EPSCs, a bipolar stimulating electrode was used to activate the neurons in the PVN with an AMFAR stimulator. The axons were recorded at a holding potential of −60 mV in the presence of 10 μM gabazine to block GABA receptor-mediated IPSCs. The evoked NMDAR-mediated EPSCs (NMDAR-EPSCs) were recorded at a holding potential of 40 mV in the presence of 10 μM gabazine and 20 μM CNQX (Li et al., 2008).

**Determination of hypoxia.** Rats were anesthetized by intraperitoneal injection of a mixture of α-chloralose (60-75 mg/kg) and urethane (800 mg/kg). Rats were mechanically ventilated via a trachea cannula connected to a rodent ventilator (CWE) with 100% O2. The exhaled CO2 concentration was monitored by a CO2 analyzer (Capstar 100; CWE) and kept at 4%-5% by adjustment of the ventilation rate (60 breaths/min) or tidal volume (−25%). The explanted hypothalamus, ABP was measured through a cannula surgically inserted into the left femoral artery. HR signal was derived from the peak interval of blood pressure pulse. For RSNA recording, a retrograde labeling of sparsely projecting PVN neurons. Sparsely projecting PVN neurons were retrogradely labeled as reported previously (Ye et al., 2011; Li et al., 2008). Rats were anesthetized with 2% isoflurane, and laminectomy was performed to expose the spinal cord at the T2-T4 level. FluorSpheres (0.04 μm; Invitrogen) or DiI (1,1’-dioctadecyl-3,3’,3’-tetramethylindocarbocyanine perchlorate, #D282, Thermo Fisher Scientific) were pressure ejected bilaterally (Nanoject II; Drummond Scientific) through a glass pipette (20-30 μm in tip diameter) inserted into the intermediolateral region of the spinal cord in 3 separate 50 nl injections on each side. After injection, rats were treated prophylactically with an antibiotic (5 mg/kg enrofloxacin, s.c., daily for 3 d) and analgesic (0.5 mg/kg buprenorphine, s.c., every 12 h for 2 d). The rats were allowed to recover for 5 d to permit FluorSpheres to be transported to the PVN. To assess the distribution of sparsely projecting neurons in the PVN, some brain sections from DiI-injected rats were coimmunolabeled with NeuN, a neuronal marker, using a rabbit anti-NeuN antibody (#ab177487, 1:50; Abcam) and an AlexaFluor-488-conjugated goat anti-rabbit secondary antibody (#a150077, 1:1000, Abcam), as described previously (Zhu et al., 2016).
branch of the left renal nerve was isolated and cut off at the distal end. The renal nerve activity was recorded using a stainless electrode, and RSNA signal was amplified (amplifier model P511; Grass Instrument). RSNA and ABP were recorded using a 1401-PLUS analog-to-digital converter and Spike2 system (Cambridge Electronic Design). The background noise was determined after rats were killed by intraperitoneal injection of an overdose of sodium pentobarbital at 200 mg/kg at the end of each experiment. The RSNA was integrated after subtraction of background noise. The basal values were obtained by averaging the signal over a 60 s period immediately before each drug treatment. Response values after each treatment were averaged over 60 s when maximal response occurred. The basal values were set as 100%, and the percentage change in RSNA from the baseline values at the same time points in respective unstressed (control) WKY and BHR.

For PVN microinjections, a glass pipette (tip diameter, 20-30 μm; Invitrogen) in the drug solutions. Rats in which the pipette tip was placed outside the PVN were excluded from data analysis. For the time course of changes in ABP and HR in WKY and BHR subjected to CUMS daily for 6 weeks. Adult BHR have been used as a model to study the role of the relationship between genetic predisposition and environmental challenges in hypertension development (Lawler et al., 1988; Fisher and Tucker, 1991; Mansi and Drolet, 1997). As expected, BHR used in our study had a baseline mean ABP (MAP) significantly higher than that of WKY but lower than that of SHR ($F_{2,99} = 19.31$, $p = 0.0093$ compared with WKY, $p = 0.0017$ compared with SHR; Table 1). Compared with no stress in WKY, CUMS gradually and significantly induced hypertension persisting after discontinuance of CUMS in BHR.

Results
CUMS induces hypertension persisting after discontinuance of CUMS in BHR
As a first step toward understanding how genetic-environment interactions influence hypertension development, we determined the time course of changes in ABP and HR in WKY and BHR subjected to CUMS daily for 6 weeks. Adult BHR have been used as a model to study the role of the relationship between genetic predisposition and environmental challenges in hypertension development (Lawler et al., 1988; Fisher and Tucker, 1991; Mansi and Drolet, 1997). As expected, BHR used in our study had a baseline mean ABP (MAP) significantly higher than that of WKY but lower than that of SHR ($F_{2,99} = 19.31$, $p = 0.0093$ compared with WKY, $p = 0.0017$ compared with SHR; Table 1). Compared with no stress in WKY, CUMS gradually and significantly increased the baseline MAP, measured during the light and dark cycles, in freely moving WKY ~4 weeks after CUMS.
was initiated (n = 9 rats per group, F[3,32] = 35.12, p = 0.013; Fig. 1A,B). After termination of CUMS, the MAP of CUMS-treated WKY rapidly returned to the baseline level within 5 d (Fig. 1A, B). However, CUMS for 6 weeks did not significantly alter the HR in WKY rats (Fig. 1C, D).

Furthermore, the MAP in CUMS-treated BHR was significantly elevated from 114.24 ± 8.22 mmHg at baseline to 143.84 ± 5.85 mmHg after 2 weeks of CUMS, and it reached 158.22 ± 59.29 mmHg after 5 weeks of CUMS (n = 9 rats, F[3,32] = 82.74, p = 0.003) (Fig. 1A, B). Strikingly, the higher MAP during the light and dark cycles in CUMS-treated BHR was persistent and remained elevated, at a level similar to that of SHR, for at least 2 weeks after termination of CUMS (Fig. 1A, B). CUMS for 6 weeks also caused a sustained increase in the HR.
during the light and dark cycles in BHR ($F_{(3,32)} = 12.13, p = 0.0070$; Fig. 1C,D). The baseline MAP and HR in unstressed BHR remained stable during the entire experimental period. These results indicate that exposure to CUMS selectively causes long-lasting elevation of ABP and HR in BHR.

CUMS persistently upregulates $\alpha_2\delta-1$ and increases synaptic expression of $\alpha_2\delta-1$-bound NMDARs in the PVN in BHR

To determine whether CUMS differentially affects synaptic expression of NMDARs and $\alpha_2\delta-1$ in the PVN in BHR and WKY, we isolated synaptosomes from PVN tissues obtained
from unstressed BHR and WKY as well as stressed BHR and WKY 1 week after discontinuance of CUMS. Immunoblotting showed that the protein level of GluN1, an obligatory subunit of NMDARs (Salussolia et al., 2011), in the PVN synaptosomes was significantly greater in CUMS-treated BHR than in CUMS-treated WKY (n = 6 rats per group, F(3,20) = 10.54, p = 0.002; Fig. 2A). Also, the α2δ-1 protein level in synaptosomes was significantly higher in CUMS-treated BHR than in CUMS-treated WKY (n = 5 rats per group, F(3,16) = 9.07, p = 0.003; Fig. 2B). In contrast, the protein levels of GluN1 and α2δ-1 in PVN synaptosomes did not differ significantly between unstressed BHR and unstressed WKY (Fig. 2A,B).

We next used real-time PCR to determine whether CUMS differentially increases the expression of GluN1 and α2δ-1 in the PVN tissue in BHR and WKY. The mRNA level of GluN1 in the PVN was similar between unstressed BHR and unstressed WKY and between CUMS-treated BHR and CUMS-treated WKY (Fig. 2C). Also, the mRNA level of α2δ-1 in the PVN did not differ significantly between unstressed BHR and unstressed WKY. However, the α2δ-1 mRNA level in the PVN was significantly higher in CUMS-treated BHR than in CUMS-treated WKY (n = 7 rats per group, F(3,22) = 13.29, p < 0.001; Fig. 2D).

We then used coimmunoprecipitation to determine whether CUMS potentiates the physical interaction between α2δ-1 and NMDARs in the PVN. The α2δ-1-GluN1 protein complex in the PVN synaptosomes was precipitated using an anti-GluN1 antibody and immunoblotted by using an anti-α2δ-1 antibody. The amount of α2δ-1–GluN1 complexes was similar between CUMS-treated WKY and unstressed WKY (Fig. 2E). In contrast, the α2δ-1–GluN1 protein level in the PVN synaptosomes was significantly greater in CUMS-treated BHR than in unstressed BHR (n = 6 rats per group, F(3,24) = 14.08, p < 0.001; Fig. 2E). These findings indicate that chronic stress persistently induces α2δ-1 upregulation and increases synaptic expression of α2δ-1–bound NMDARs in the PVN in BHR selectively. To determine the synaptic mechanisms involved in chronic stress-induced persistent hypertension, we conducted the following experiments in unstressed BHR and stressed BHR 7-12 d after discontinuing CUMS.

CUMS induces α2δ-1–dependent activation of presynaptic NMDARs in PVN presynaptic neurons in BHR Presynaptic NMDARs in the PVN are not functionally active in normotensive conditions, but in SHR, they become tonically...
activated to increase synaptic glutamate release (Li et al., 2008; Ma et al., 2018b). We thus determined whether CUMS potentiates presynaptic NMDAR activity in PVN presympathetic neurons in BHR. We recorded mEPSCs in retrogradely labeled, spinally projecting PVN neurons (Fig. 3A) in brain slices from stressed BHR 1 week after discontinuance of CUMS and from un-stressed BHR. The baseline frequency of mEPSCs, but not the amplitude, was significantly higher in labeled PVN neurons in CUMS-treated BHR than in unstressed BHR (n = 11 neurons in stressed BHR, n = 10 neurons in unstressed BHR, t19 = 2.13, p = 0.041; Fig. 3B,C,E). Bath application of AP5 (50 μmol/L), a specific NMDAR antagonist, significantly decreased the frequency of mEPSCs in CUMS-treated BHR (n = 11 neurons, F(2,30) = 14.50, p < 0.001; Fig. 3C,E). However, AP5 application had no effect on the baseline frequency or amplitude of mEPSCs in labeled PVN neurons in unstressed BHR (Fig. 3B,E). These results suggest that CUMS causes tonic activation of presynaptic NMDARs, increasing glutamatergic inputs to PVN presympathetic neurons in BHR.

We next determined whether α2δ−1 is involved in tonic activation of presynaptic NMDARs in PVN presympathetic neurons in stressed BHR. We treated brain slices with gabapentin (100 μmol/L for 30-60 min), an α2δ−1 inhibitory ligand (Gee et al., 1996; J. Chen et al., 2018). Gabapentin treatment significantly reduced the increased mEPSC frequency in labeled PVN neurons in CUMS-treated BHRs (n = 9 neurons, F(5,54) = 10.04, p = 0.004; Fig. 3D,E). Subsequent bath application of AP5 did not change this reduced mEPSC frequency by gabapentin (Fig. 3D,E), suggesting that α2δ−1 is involved in CUMS-induced presynaptic NMDAR activation in PVN presympathetic neurons in BHR.

We then determined whether α2δ−1−bound NMDARs mediate increased synaptic glutamate release to PVN presympathetic neurons in CUMS-treated BHR. We treated hypothalamic slices from CUMS-treated BHR with Tat-fused α2δ−1C terminus peptide (α2δ−1Tat peptide), which can penetrate the cell membrane and specifically disrupt the α2δ−1−NMDAR interaction (J. Chen et al., 2018). Treatment with α2δ−1Tat peptide (1 μmol/L) for 30-60 min (Ma et al., 2018b) significantly decreased the frequency, but not the amplitude, of mEPSCs in labeled PVN neurons in CUMS-treated BHR (n = 13 neurons, F(5,51) = 24.68, p < 0.001; Fig. 4A-D). Subsequent bath application of AP5 (50 μmol/L) had no effect on the reduced frequency of mEPSCs in these neurons. In contrast, treatment with Tat-fused control peptide (1 μmol/L for 30-60 min) did not significantly alter the higher frequency of mEPSCs in labeled PVN neurons in CUMS-treated BHR; AP5 application still significantly reduced the mEPSC frequency in these neurons (n = 13 neurons; Fig. 4D). These data indicate that presynaptic α2δ−1−bound NMDARs are essential for CUMS-potentiated glutamate release to PVN presympathetic neurons in BHR.
CUMS augments postsynaptic NMDAR activity via \( \alpha_{2\delta}-1 \) in PVN presympathetic neurons in BHR

Furthermore, we determined whether CUMS alters postsynaptic NMDAR activity in PVN presympathetic neurons in BHRs. We recorded NMDAR-EPSCs and AMPAR-EPSCs of spinally projecting PVN neurons evoked via focal stimulation (Li et al., 2008) in CUMS-treated BHR 1 week after termination of CUMS and in unstressed BHR. The amplitude of evoked AMPAR-EPSCs in labeled PVN neurons was similar between CUMS-treated and unstressed BHRs (\( n = 13 \) neurons per group; Fig. 5A,C). However, the amplitude of evoked NMDAR-EPSCs was much larger in CUMS-treated BHR than in unstressed BHR (\( n = 13 \) neurons per group, \( F_{(2,36)} = 46.41, p < 0.001; \) Fig. 5A,B).

Treatment with 100 \( \mu \text{mol/L} \) gabapentin in brain slices from CUMS-treated BHR had no effect on the amplitude of evoked AMPAR-EPSCs, but it normalized the increased amplitude of evoked NMDAR-EPSCs in labeled PVN neurons (\( n = 13 \) neurons per group; Fig. 5A-C). In addition, treatment with 1 \( \mu \text{mol/L} \) \( \alpha_{2\delta}-1 \) Tat peptide significantly decreased the amplitude of evoked NMDAR-EPSCs, but not evoked AMPAR-EPSCs, in labeled PVN neurons in CUMS-treated BHR (\( n = 13 \) neurons per group, \( t_{(24)} = 6.03, p < 0.001; \) Fig. 5D,E). However, treatment with 1 \( \mu \text{mol/L} \) control peptide had no effect on the amplitude of evoked NMDAR-EPSCs or AMPAR-EPSCs in labeled PVN neurons in CUMS-treated BHR (Fig. 5D,E). Together, these results suggest that CUMS causes \( \alpha_{2\delta}-1 \)-dependent potentiation of postsynaptic NMDAR activity in PVN presympathetic neurons in BHR selectively.
CUMS-induced increase in sympathetic nerve discharges is maintained by NMDARs in the PVN in BHR

To functionally relate CUMS-induced synaptic plasticity in BHR to regulation of sympathetic outflow in vivo, we recorded RSNA in unstressed BHR and CUMS-treated BHR 1 week after terminating CUMS. The basal ABP and HR were significantly greater in CUMS-treated BHR than in unstressed BHR (Fig. 6). Also, the baseline of integrated RSNA (averaged over 60 s) was significantly higher in CUMS-treated BHR than in unstressed BHR (0.160 ± 0.025 mV/s vs 0.065 ± 0.015 mV/s, t(15) = 3.18, p = 0.006). In unstressed BHR, bilateral microinjection of AP5 (1.0 nmol, 50 nl) (Ma et al., 2018b) into the PVN had no significant effect on the MAP, HR, or RSNA (Fig. 6A,C-E). However, in CUMS-treated BHR, bilateral microinjection of the same amount of AP5 into the PVN normalized the MAP, HR, and RSNA from elevated baseline levels to the levels in unstressed BHR (n = 8 rats in each group; Fig. 6B-F). These data suggest that the CUMS-induced persistent hypertension in BHR results from excess sympathetic drive, which is sustained by NMDAR activity in the PVN.

$\alpha_{2\delta}-1$-bound NMDARs in the PVN mediate the CUMS-induced increase in sympathetic outflow in BHR

Next, we determined the role of $\alpha_{2\delta}-1$-bound NMDARs in the CUMS-induced increase in sympathetic outflow in CUMS-treated BHR. We microinjected $\alpha_{2\delta}-1$Tat peptide or control peptide and then injected AP5 into the PVN in CUMS-treated BHR 1 week after discontinuing CUMS. In these animals, bilateral microinjection of control peptide (50 pmol, 50 nl) had no effect on the MAP, HR, or RSNA, and subsequent microinjection of AP5 (1.0 nmol, 50 nl) significantly decreased MAP, HR, and RSNA (n = 5 rats; Fig. 7A,C,D). In contrast, microinjection of $\alpha_{2\delta}-1$Tat peptide (50 pmol, 50 nl) alone into the PVN significantly reduced the MAP (F(3,24) = 6.94, p = 0.0311), HR (F(3,24) = 8.13, p = 0.015), and RSNA (F(3,24) = 6.48, p = 0.0189) from baseline levels (n = 7 rats; Fig. 7B-D). When the inhibitory effect of $\alpha_{2\delta}-1$Tat peptide reached maximum, microinjection of AP5 did not further reduce MAP, HR, or RSNA (Fig. 7B-E). These findings suggest that $\alpha_{2\delta}-1$-coupled NMDARs in the PVN maintain the CUMS-induced hyperactivity of the sympathetic nervous system in BHR.
Fig. 8. Systemic administration of memantine or gabapentin decreases CUMS-induced persistent hypertension in conscious BHR. Mean data show the time course of changes in MAP after a single intraperitoneal injection of gabapentin (50 mg/kg) or memantine (5 mg/kg) in unstressed control BHR and CUMS-treated BHR. ABP was measured using telemetry in conscious rats. Data are mean ± SEM. Repeated-measures ANOVA with the Dunnett post hoc test (n = 9 rats per group). **p < 0.01, ***p < 0.001, compared with the respective baseline (time 0 min) before drug injection in the same group.

Systemic administration of memantine or gabapentin reduces CUMS-induced persistent hypertension in conscious BHR.

In discussion, to ascertain the potential clinical relevance of our findings, we examined the effects of systemically administered memantine, an FDA-approved NMDAR antagonist for treating Alzheimer disease (Farlow and Cummings, 2007), and gabapentin on the persistently elevated ABP, measured using implanted radiotelemetry, in freely moving, conscious BHR 1 week after terminating CUMS. The effective doses of gabapentin and memantine in rodents have been determined previously (S. R. Chen et al., 2009, 2014). In 9 unstressed BHR, intraperitoneal injection of 5 mg/kg memantine had no significant effect on MAP compared with the baseline level (Fig. 8). However, in CUMS-treated BHR, injection of memantine significantly decreased MAP from its CUMS-elevated baseline, and this inhibitory effect lasted for ~90 min (n = 9 rats, F(8,40) = 21.10, p < 0.001; Fig. 8). Similarly, intraperitoneal administration of 50 mg/kg gabapentin significantly reduced MAP from its baseline in CUMS-treated BHR but not in unstressed BHR (n = 9 rats per group, F(8,40) = 38.40, p < 0.001; Fig. 8). Thus, memantine and gabapentin are effective in attenuating chronic stress-induced persistent hypertension in BHR.

Discussion

The major finding of our study is that BHR subjected to CUMS developed a sustained and sympathetically mediated increase in ABP long after CUMS was discontinued. The increase in ABP in CUMS-treated BHR reached the level of higher ABP seen in SHR. In contrast, in normotensive WKY, the increase in ABP elicited by CUMS quickly returned to the control level after CUMS was discontinued. Previous studies show that BHR subjected repeatedly to the same physical or psychosocial stressor do not reliably develop chronic hypertension (Harrap et al., 1984; Cox et al., 1985; Fisher and Tucker, 1991). To avoid stressor adaption, we used a CUMS paradigm, which effectively mimics depression and anxiety in humans (Willner, 2017). In our study, a significant increase in the MAP occurred after 4 weeks of CUMS in normotensive WKY rats. Others have shown that chronic mild stress for 3 weeks does not significantly increase the MAP in normotensive rats (Grippo et al., 2002; Schaeuble et al., 2019).

To date, brain regions involved in CUMS-induced chronic hypertension have not been specifically identified. Our study provides direct evidence that CUMS-induced chronic hypertension in BHR is primarily maintained by excess sympathetic outflow emanating from the PVN. Thus, the PVN represents a key brain region where the hereditary and environmental conditions interact to cause persistent hypertension.

Our study provides the first evidence that CUMS potentiates NMDAR activity, at both presynaptic and postsynaptic sites, in the PVN, which constitutes a sustained excitatory drive for sympathetic output in BHR. We showed that the increased synaptic NMDAR activity in PVN presynaptic neurons coincides with persistent hypertension in BHR after discontinuance of CUMS. Furthermore, blocking NMDARs in the PVN attenuated elevated ABP and sympathetic nerve discharges in CUMS-treated BHR but not in unstressed BHR. The precise mechanism under which CUMS increases synaptic NMDAR activity in the PVN in BHR is uncertain. Increased NMDAR phosphorylation by various protein kinases can potentiate synaptic NMDAR activity in PVN presynaptic neurons in SHR (Ye et al., 2011; Li et al., 2015; Qiao et al., 2017; Ma et al., 2019). Chronic stress increases levels of circulating corticosterone and angiotensin II (Yang et al., 1993; Lowrance et al., 2016) and angiotensin AT1 receptor expression in the PVN (Dumont et al., 1999). Both stress hormones may augment NMDAR activity in the hippocampus and PVN (Glass et al., 2015; Mikasova et al., 2017; Zhou et al., 2018; Ma et al., 2019). Although our present study is focused on chronic stress-induced hypertension in BHR, a similar mechanism likely applies to stress-induced transient hypertension in normotensive animals. In this regard, CUMS induces a significant increase in synaptic NMDAR activity in the PVN in Sprague Dawley rats (Zhou et al., 2018). Our findings demonstrate that CUMS can impact NMDAR-mediated synaptic plasticity in the hypothalamus, which dictates sympathetic output in BHR, thus providing a molecular framework for understanding the interplay between genetic and environmental cues in hypertension development.

Another important finding of our study is that α2δ-1 is essential for CUMS-augmented synaptic NMDAR activity in the PVN and sympathetic outflow in BHR. We showed that CUMS-induced persistent hypertension is associated with increased synaptic expression of GluN1 but not the mRNA level of GluN1, suggesting that increased NMDAR synaptic trafficking likely accounts for increased synaptic NMDAR activity in the PVN in CUMS-treated BHR. Others have also reported that chronic stress does not alter the GluN1 mRNA level in the PVN, measured using ISH (Ziegler et al., 2005). Recent studies revealed that α2δ-1 functions as an auxiliary protein of NMDARs and is required for NMDAR synaptic trafficking in the PVN potentiated by angiotensin II and in SHR (Ma et al., 2018a,b). In this study, we found that CUMS increased synaptic expression of α2δ-1 and the physical interaction of α2δ-1 with NMDARs in the PVN in BHR. Importantly, we showed that interrupting the α2δ-1–NMDAR interaction with α2δ-1 Tat peptide reversed sympathetic nerve discharges and synaptic NMDAR activity in the PVN potentiated by CUMS in BHR. Because angiotensin II increases the α2δ-1–NMDAR interaction in the PVN (Ma et al., 2018a), chronic stress–augmented angiotensin II levels may be involved in the α2δ-1–dependent increase in synaptic NMDAR activity in BHR. Also, increased NMDAR phosphorylation can enhance the physical interaction between α2δ-1 and NMDARs in the spinal cord (Huang et al., 2020). CUMS-induced increases
in neuronal excitability and protein kinase activity in the PVN.

We showed that CUMS-induced persistent hypertension is associated with an increased mRNA level of α2δ-1 in the PVN in BHR. Thus, the increased availability of α2δ-1 protein could promote its coupling to NMDARs and the synaptic trafficking of α2δ-1-bound NMDARs in the PVN in CUMS-treated BHR. Chronic stress likely impacts epigenetic/transcriptional regulation of α2δ-1 and thus controls synaptic NMDAR activity in the PVN and sympathetic output in subjects with genetic predisposition to hypertension. Further studies are needed to define how chronic stress alters epigenetic reprogramming to upregulate α2δ-1 and other hypertension-predisposing mediators, such as NKCC1 and angiotensin AT1 receptors (Ye et al., 2012; Ma et al., 2019), in the hypothalamus of BHR.

In conclusion, our study identifies α2δ-1-bound NMDARs in the PVN as a target and effector of genetic–environment interactions that drive excess sympathetic output and persistent hypertension. Understanding the mechanisms by which chronic stress promotes hypertension development can enable the design of rational strategies to treat persistent hypertension. Our study suggests that α2δ-1-bound NMDARs could be targeted to treat chronic hypertension caused by stress. This notion is supported by our findings that memantine and gabapentin, two clinically used drugs that inhibit NMDARs and α2δ-1, respectively, similarly reversed CUMS-induced persistent hypertension in BHR. Clinical studies show that both memantine and gabapentin reduce anxiety (Lavigne et al., 2012; Schwartz et al., 2012) and that gabapentinoids attenuate tracheal intubation–induced pressor responses (Fassoulaki et al., 2006; W. Chen et al., 2019). Nevertheless, the therapeutic effects of memantine and gabapentinoids for treating neurogenic, resistant hypertension need to be validated in future clinical studies.

References

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