RESEARCH ARTICLE

Chronic stimulation of group II metabotropic glutamate receptors in the medulla oblongata attenuates hypertension development in spontaneously hypertensive rats

Julia Chu-Ning Hsu, Shin-ichi Sekizawa *, Ryota Tochinai, Masayoshi Kuwahara *

Department of Veterinary Pathophysiology and Animal Health, Graduate School of Agricultural and Sciences, The University of Tokyo, Tokyo, Japan

* akuwam@mail.ecc.u-tokyo.ac.jp (MK); a-ssekiz@mail.ecc.u-tokyo.ac.jp (SS)

Abstract

Baroreflex dysfunction is partly implicated in hypertension and one responsible region is the dorsal medulla oblongata including the nucleus tractus solitarius (NTS). NTS neurons receive and project glutamatergic inputs to subsequently regulate blood pressure, while G-protein-coupled metabotropic glutamate receptors (mGluRs) play a modulatory role for glutamatergic transmission in baroreflex pathways. Stimulating group II mGluR subtype 2 and 3 (mGluR2/3) in the brainstem can decrease blood pressure and sympathetic nervous activity. Here, we hypothesized that the chronic stimulation of mGluR2/3 in the dorsal medulla oblongata can alleviate hypertensive development via the modulation of autonomic nervous activity in young, spontaneously hypertensive rats (SHRs). Compared with that in the sham control group, chronic LY379268 application (mGluR2/3 agonist; 0.40 \( \mu \)g/day) to the dorsal medulla oblongata for 6 weeks reduced the progression of hypertension in 6-week-old SHRs as indicated by the 40 mmHg reduction in systolic blood pressure and promoted their parasympathetic nervous activity as evidenced by the heart rate variability. No differences in blood catecholamine levels or any echocardiographic indices were found between the two groups. The improvement of reflex bradycardia, a baroreflex function, appeared after chronic LY379268 application. The mRNA expression level of mGluR2, but not mGluR3, in the dorsal medulla oblongata was substantially reduced in SHRs compared to that of the control strain. In conclusion, mGluR2/3 signaling might be responsible for hypertension development in SHRs, and modulating mGluR2/3 expression/stimulation in the dorsal brainstem could be a novel therapeutic strategy for hypertension via increasing the parasympathetic activity.

Introduction

Hypertension is a leading risk factor for cardiovascular diseases worldwide [1], and blood pressure (BP) control is important in reducing the risk of these illnesses [2]. Hypertensive patients
are consistently cautioned to exercise regularly, manage their diet, and take antihypertensive medication [1]. Although hypertension treatment involves various antihypertensive drugs including alpha- and beta-adrenergic blockers, diuretics, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, vasodilators, and/or calcium-channel blockers [3], these remain unsatisfactory.

Baroreflex function properly maintains heart rate (HR) and BP within a physiological range to support the whole-body system for active daily life [4], and its pathways are greatly involved in medulla oblongata areas. Among which, the nucleus tractus solitarii (NTS) is the first synaptic site to receive afferent information from glutamergic peripheral baroreceptors and airway receptors [5, 6]. NTS neurons send glutamatergic projections to neurons located in the ipsilateral caudal ventrolateral medullary depressor area (CVLM), which then send inhibitory gamma-aminobutyric acid (GABA)ergic projections to neurons located in the ipsilateral ventrolateral medullary pressor area (RVLM) [7]. Activation of second- and higher-order baroreceptor NTS neurons excites the CVLM neurons, which in turn inhibit the sympathoexcitatory RVLM neurons. These actions finally elicit depressor and bradycardic responses. G-protein-coupled metabotropic glutamate receptors (mGluRs), which provide acute and long-term modulation of glutamatergic transmission in various neural networks [8–10], are also present in the medulla oblongata, particularly in the NTS [11, 12]. Microinjection of mGluR modulators into the dorsal area of medulla oblongata has been previously attempted with Sprague-Dawley or Wistar rats for short-term administration. Of these modulators, agonists for group II mGluR subtypes 2 and 3 (mGluR2/3) could acutely change HR, BP, and sympathetic nervous activity [7, 13–16], but these results show discrepancies.

Spontaneously hypertensive rats (SHRs) have been widely used as a model animal of essential hypertension for studies on the mechanism of this hypertension [17]. The HR and BP of SHR and Wistar Kyoto rats (WKYs) were nearly the same until the age of 2 weeks [18]. Thereafter, however, the HR of SHRs becomes faster from the age of 3 weeks and the BP of SHRs becomes slightly higher at the age of 4 weeks compared to WKYs [19]. Meanwhile, the firing rates of the extracellular units of RVLM neurons are higher in adult SHRs than WKYS in vivo [20]. The electrophysiological properties of RVLM neurons and their responses to angiotensin II differ between WKYS and SHRs, even in the neonatal stage [21]. mGluRs, especially postsynaptic ones, do not exhibit any function at younger (juvenile) stages in some cases [22–24] but regulate various neuronal functions at different developmental stages [25–34]. Furthermore, mGluR2/3 agonists hyperpolarize neurons in normotensive rats [35], while the agonists effects seemed to be attenuated in adult SHRs (authors’ observation). In this work, we hypothesized that the chronic stimulation of mGluR2/3 in the dorsal medulla oblongata, especially NTS, of juvenile SHRs might alleviate the development of hypertension by modulating the autonomic nervous activities. For hypothesis testing, a selective mGluR2/3 agonist, LY379268, was added to the extracellular cerebrospinal fluid of the brainstem with a mini-osmotic pump for the chronic agonist treatment. HR and BP were measured by the tail-cuff method throughout the developmental stages of hypertension, and autonomic nervous activity was evaluated by the power spectral analysis of heart rate variability (HRV) using a radio-telemetry system.

Materials and methods

Animals

All experimental protocols were approved by The Animal Care and Use Committee of the University of Tokyo (No. P17-033). All animals were managed according to the Guidelines for the Care and Use of Laboratory Animals established by the Graduate School of Agriculture and Life Sciences at the University of Tokyo.
Four-week-old male SHRs and WKYs were purchased from Charles River Laboratories Japan, Inc. (Yokohama, Japan) and housed in a temperature-controlled room (24 ± 1˚C) under an automatic controlled lighting (light on: 8:00–20:00) with access to food and water ad libitum.

**Dorsal hindbrain mGluR2/3 treatment**

Isoflurane was used as anesthetic (Pfizer Japan Inc., Tokyo, Japan), and the following surgical procedures were performed under a specific level of anesthesia, i.e., the absence of withdrawal reflexes. Animals aged 6 weeks were implanted with a mini-osmotic pump (ALZET Model 2006, DURECT Corporation, Cupertino, CA, United States) that can continuously deliver solutions for 6 weeks of treatment, and the pump was removed at the end of the treatment. For the implantation, the lateral cervical space was opened, and a catheter (external diameter 0.61 mm, internal diameter 0.28 mm) was inserted in the cranial cavity through the foramen magnum. Its tip was located near the caudal end of the medulla oblongata, and its other end was connected to the mini-osmotic pump in the subcutaneous area of the back, which was filled with LY379268 ((1R,4R,5S,6R)-4-Amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid) (0.40–40.0 μg/day) at least 60 hours prior to implantation. Sham surgery without mini-osmotic pump was performed on control rats.

**Measurement of HR and BP**

The HR and BP of conscious animals were measured with the tail-cuff apparatus (BP-98AL, Softron Co., Ltd., Tokyo, Japan) at 3 days before surgery and every week in 7- to 18-week-old rats. Details of the operating procedures can be found in previous paper [36].

**Echocardiographic and renal ultrasonographic measurements**

Echocardiography and renal ultrasonography tests were performed in 18-week-old SHRs under 2% isoflurane anesthesia in air with flow rate of 1 l/min by using a preclinical imaging system (Vevo 3100, FujiFilm VisualSonics, Toronto, ON, Canada) and a linear array transducer (MS-550S, FujiFilm VisualSonics, Toronto, ON, Canada). Echocardiography was recorded using the preclinical imaging system [37]. Image analysis was performed for left-ventricular short-axis, left-ventricular inflow waveform, and mitral valve septal tissue waveform. Cardiac function parameters such as HR, ejection fraction (EF), and cardiac output (CO) were calculated using an analysis software (Vevo LAB, FujiFilm VisualSonics, Toronto, ON, Canada). The parameters of renal arteries on both sides, especially resistive index (RI) and pulsatility index (PI) that indicate the severity of renal diseases, were also examined and calculated. A RI higher than 0.75 or a PI higher than 1.55 indicates chronic renal failure [38].

**Implantation of telemetry device for electrocardiography recording**

In addition to the mini-osmotic pump, an ECG telemetry device (ATE-01S, Softron Co., Ltd., Tokyo, Japan) was implanted in the backs of 6-week-old SHRs under isoflurane anesthesia. Paired wire electrodes of the transmitter were subcutaneously placed on the dorsal and ventral thorax to record the apex-base lead ECG. Recordings were started a week after surgery with a signal receiving board (ATR-1001, Softron Co., Ltd., Tokyo, Japan) placed underneath each cage in the chamber (MIR-554, Panasonic, Japan) with standardized temperature (24˚C) and lighting (8:00–20:00). An ECG processor system (Softron Co., Ltd., Tokyo, Japan) was used to continuously record ECG signals.
HRV analysis

Power spectral analysis of HRV was performed as previously described [39, 40]. Time- and frequency-domain methods were used to assess autonomic nervous activity. The time-domain analysis was based on R-R intervals for calculating standard deviation (SD) and coefficient of variation (CV), which are regarded as the indices of parasympathetic activity [40]. In the frequency-domain method, power spectral components were primarily classified into low (LF; 0.04 to 1.0 Hz) and high (HF; 1.0 to 3.0 Hz) frequency ranges as different elements of autonomic nervous activities [39]. The normalized power spectral components of low frequency and high frequency (LFnu and HFnu, respectively) were also calculated to diminish the influence of very low frequency and highlight the interaction between sympathetic and parasympathetic nerves [41, 42]. LF is affected by sympathetic and parasympathetic nervous activities, HF is as an index of the parasympathetic nervous activity, and the ratio of LF to HF (LF/HF) is as an index of the balance of autonomic nervous system [39].

Measurement of catecholamine concentration

In brief, 12-week-old SHRs were decapitated under isoflurane anesthesia, and blood samples were collected from the left superior vena cava. The catecholamine concentration in blood serum was assessed with an ELISA kit (Cat Combi ELISA RUO EIA-4309R, DRG Instruments GmbH, Marburg, Germany) in accordance with the manufacturer’s instructions.

Assessment of baroreflex sensitivity

After dorsal hindbrain mGluR2/3 treatment was completed at 12 weeks of age, implants were removed, and SHRs were subjected to invasive catheterization. They were anesthetized with an intraperitoneal injection of urethane (1.5 g/kg) dissolved in distilled water. The animals were placed in the supine position, and then the following surgical procedures were performed after confirming the abolition of pain reflexes induced by pinching their paws. A polyethylene catheter was inserted into the left femoral vein for intravenous administration of pharmacological agents. The left femoral artery was also cannulated to measure arterial BP.

Arterial BP was tracked via the catheterized femoral artery with a catheter-transducer system (Nihon Kohden, Tokyo, Japan) connected to a computer acquisition system (Softron Co., Ltd., Tokyo, Japan) during the entire measurement. The mean arterial pressure (MAP) was calculated from measured systolic and diastolic BP. Limb lead II electrocardiogram was recorded with needle electrodes. After 5 min of basal control recording, intravascular injections of phenylephrine (PE; 21 μg/kg) or sodium nitroprusside (SNP; 50 μg/kg) were performed through the catheter of the femoral vein [43, 44]. Cardiovascular waveforms were then recorded for 15 min. After eliminating interference, HR and MAP were measured with averaged of each recording minute.

Baroreflex sensitivity was evaluated with changes in MAP (ΔMAP) and corresponding HR reflex (ΔHR) at the peak responses to PE and SNP application [43, 45]. The baseline data for comparing peak responses were calculated as the average values over the 5-min period of basal value recording. PE and SNP increase or decrease MAP accordingly, so HR responses are defined as bradycardia and tachycardia reflex, respectively [43, 46].

RNA isolation and quantitative real-time Polymerase Chain Reaction (PCR)

Sham control and LY379268 treated groups of SHRs and WKYS were decapitated at 21 weeks of age under deep isoflurane anesthesia. Sham control groups of SHRs had developed sufficient
hypothesis at that age. Whole medulla oblongata specimens were dissected, and total RNA was isolated using TRIzol reagent (Gibco-BRL, Grand Island, NY, United States). First-strand cDNA was synthesized using SuperScript IV VILO Master Mix with ezDNase Enzyme (Invitrogen, Carlsbad, CA, United States). With cDNA as a template, real-time PCR was performed with THUNDERBIRD SYBR qPCR Mix (Toyobo, Osaka, Japan) and LightCycler (Roche, Mannheim, Germany). The following primers for real-time PCR were designed based on published sequences: rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH; an internal control) forward primer, 5'-TCA CCA TGG AGA AGG -3'; reverse primer, 5'-GCT AAG CAG TTG GTG CA-3'; rat mGluR2 forward primer, 5'-CGT GAG TTC TGG GAG AG-3'; reverse primer, 5'- GCG GAC CTC ATC GTC AGT AT-3'; rat mGluR3 forward primer, 5'-GTG GTC TTG GGC TGT TTG TT-3'; reverse primer, 5'-GCA TGT GAG CAC TTT GT-3'.

Statistical analysis
All data are expressed as the mean ± SEM. HRV data were averaged from 24-hour recordings, or 12-hour recordings of either light or dark photoperiod. Prism 8 software (Graphpad Software Inc., San Diego, CA, United States) was used for all statistical analysis. Dose-dependent hypertensive effect was evaluated by one-way ANOVA, followed by Tukey’s test. The Kaplan–Meier curve and the log-rank test were used to examine SHR mortality rate difference among different doses of LY379268 treatment. The dose and number of deaths after LY379268 treatment were used to calculate the 50% lethal dose (LD₅₀) (Quest Graph™ LD₅₀ Calculator, AAT Bioquest, Inc., Sunnyvale, CA, United States). HR and BP between WKYS and SHRs during LY379268 treatment were evaluated through two-way ANOVA, followed by Tukey’s test for multiple comparisons. Statistical analyses were performed using unpaired t-test to examine other types of data. Significant differences were considered at p < 0.05.

Results
Five groups of 6-week-old SHRs received five different doses of LY379268. Within a week after surgical operation, systolic BP (SBP) seemed to be reduced for the 0.40 μg/day dose, but had the tendency of getting close to control values with higher doses. However, no significant difference was found between any two doses (P = 0.30) (Fig 1A). Kaplan–Meier survival with a follow-up period revealed that the SHRs receiving 0.40 μg/day LY379268 showed the best survival rate without death as confirmed by the log-rank test (Fig 1B, P < 0.05), although LY379268 exhibited systemic toxicity (e.g., losing weight dramatically and less capability of action to meet endpoints for euthanasia) (LD₅₀ = 4.63 μg/day) when administered to the dorsal medulla oblongata of SHRs (Fig 1C). Therefore, 0.40 μg/day was selected for following studies to achieve the same level of effect on BP.

Effects of LY379268 (0.40 μg/day) on BP and HR in hypertensive development
Fig 2 shows time course changes of SBP, diastolic BP (DBP), and heart rate (HR) in 6- to 18-week-old SHRs. SBP and DBP increased with age in SHRs but not in WKYS regardless of treatment (Fig 2A and 2C). After LY379268 (mGluR2/3 agonist) treatment ended at 12 weeks of age, the agonist effect on SBP was evident in SHRs (sham control vs. agonist, 203.9 ± 2.1 vs. 160.4 ± 14.7 mmHg; P < 0.001) but not in WKYS (sham control vs. agonist, 123.5 ± 3.1 vs. 111.4 ± 5.3 mmHg; P = 0.62) (Fig 2B). This effect persisted at 18 weeks of age, 6 weeks after the absence of treatment (sham control vs. agonist, 202.9 ± 6.0 vs. 163.7 ± 4.8 mmHg; P < 0.001). The agonistic effect on DBP was not evident in SHRs even at 12 weeks of age (sham control vs.
A

SBP (mmHg) vs Dose (µg/day)

* Significant difference

B

Survival probability (%) vs Weeks after operation

- sham control
- 0.40 µg/day
- 1.3 µg/day
- 4.0 µg/day
- 10 µg/day
- 40 µg/day

C

Probability of death (%) vs Log [LY379268 dose] (µg/day)

\[ LD_{50} = 4.63 \text{ µg/day} \]
agonist, 124.9 ± 21.8 vs. 116.5 ± 14.3 mmHg; \( P = 1.0 \) (Fig 2D). DBP decreased in SHRs at 18 weeks of age, 6 weeks after the treatment ended, but the difference was not statistically significant (sham control vs. agonist, 138.9 ± 13.1 vs. 108.1 ± 10.8 mmHg; \( P = 0.082 \)) (Fig 2D). As
with SBP, LY379268 had no significant effect on DBP in WKYs at any age. HR was the same for all groups during the treatment period. After the treatment, HR was significantly higher in SHRs than WKYs regardless of the treatment dose (Fig 2E). The agonist effect of LY379268 on HR was not observed in either SHRs or WKYs even at 18 weeks of age (Fig 2F).

Echocardiography and renal ultrasonography

Representative echocardiogram recordings are shown in Fig 3, and their parameters are summarized in Table 1. Neither HR nor stroke volume was changed by mGluR2/3 agonist treatment in SHRs as indicated by the similar cardiac output among groups (Table 1). In renal studies, LY379268 significantly increased the right renal artery-lowest diastolic velocity (RRA-LDV) ($P = 0.038$) but had no effect on the left side (LRA-LDV) ($P = 0.62$) (Table 1). Other parameters such as renal artery-peak systolic velocity, RI, and PI were not affected by LY379268 treatment in the renal artery of either side. However, the RI (sham control vs. agonist, $0.74 \pm 0.04$ vs. $0.68 \pm 0.13$) and PI (sham control vs. agonist, $1.48 \pm 0.16$ vs. $1.28 \pm 0.46$) in the right renal artery nearly reached chronic renal failure levels in the sham control group compared to the LY379268 group.

Effects of LY379268 on HRV

HRV analysis revealed the effect of LY379268 on autonomic nervous function in conscious SHRs (Fig 4). HR decreased with aging, whereas the HRV indices of time- and frequency-domain analyses, namely, SD, CV, LF, and HF, time-dependently increased regardless of LY379268 treatment (Fig 4A). Compared with sham control, the LY379268-treated groups showed progressively increased LF (Fig 4G) and HF (Fig 4B) but decreased LF/HF ratio (Fig 4D) during the application period. The results of HRV analysis at 12 weeks of age in light and dark phases for 24 hours are presented in Fig 5. HR increased during the dark phase compared to the light phase in agonist and control groups (Fig 5A). Meanwhile, photoperiodic differences were not evident in HRV parameters obtained from either time- (SD and CV) or frequency- domain analysis (HF). LY379268 treatment had no effect on the time-domain data at 12 weeks of age (Fig 5C and 5E). For the parameters from frequency-domain analyses, especially in the light phase, HF (Fig 5B) and HFnu (Fig 5H) significantly increased, and the LF/HF ratio was significantly decreased (Fig 5D). The treatment effect on LF was completely opposite when the data were normalized by total power. A significant increase was found in LF (Fig 5G), whereas a significant decrease was observed in LFnu, especially in the light phase (Fig 5F). Given that spectral power in each range can be influenced by power in other ranges, such as the very low frequency range in this study, the latter result could be legitimate [41]. For the parameters from the frequency-domain analyses, the altered level of LF/HF ratio (Fig 5D) was dependent on changes in HF (Fig 5B), particularly in the light phase. In summary, mGluR2/3 agonist administration in the medulla oblongata simultaneously activates the parasympathetic nervous system and suppresses sympathetic nerve activity [39].

Effect of LY379268 on catecholamine concentration in hypertensive development

The LY379268 treatment did not change the catecholamine, adrenaline (sham control vs. agonist, $1.07 \pm 0.19$ vs. $1.15 \pm 0.32$ ng/mL; $n = 4$ or 5 each; $P = 0.70$), and noradrenaline (sham control vs. agonist, $0.83 \pm 0.25$ vs. $1.16 \pm 0.42$ ng/mL; $n = 4$ or 5 each; $P = 0.25$) levels in the blood of SHRs (Fig 6).
Effect of LY379268 on baroreflex function

The recording of HR and BP over time revealed that responses to PE or SNP exhibited a unimodal pattern (Fig 7). The initial phase had an increase in MAP (Fig 7B) and a related decrease in HR (Fig 7A) within 20 s after PE injection. The reformed phase had a sustained decrease in MAP and correlative varied HR reflex during the remaining 15-min observation period (Fig 7A and 7B). After PE injection, the MAP of both groups almost returned to the 5-min basal level, but not HR. Compared with the HR in the 5-min basal control recording, the HR of the LY379268 group was lower, while that of the sham control group was higher. After 20s from SNP injection, the primary phase showed decreased MAP (Fig 7E) and increased HR (Fig 7D). During the remaining 15-min observation period, the reformed phase had steady MAP and HR (Fig 7D and 7E). Both groups had higher level of MAP after SNP injection. The HR of the sham control group was not altered after SNP injection, while the HR of the LY379268 group showed a slight decrease. In the evaluation of baroreflex function, the LY379268 group displayed better bradycardia reflex in response to PE injection than sham control group (Fig 7C), which means that the baroreflex function of SHRs was largely augmented by LY379268 treatment. Nevertheless, both groups displayed similar levels of tachycardia reflex after SNP injection (Fig 7F).

mGluR2/3 expression in medulla oblongata

As we stated in the introduction, we have observed the NTS neurons in SHRs showing a blunted response to mGLuR2/3 compared to those in the normotensive rats. Thus, we have

Table 1. Effects of LY379268 in the brain stem on the cardiac and renal parameters of ultrasonography.

| Parameter           | Unit       | Sham control | LY379268     |
|---------------------|------------|--------------|--------------|
| Cardiac             |            |              |              |
| Heart Rate          | bpm        | 284.0±16.0   | 308.1±48.3   |
| Stroke Volume       | μL         | 217.6±10.2   | 206.2±30.0   |
| Ejection Fraction   | %          | 58.1±8.4     | 65.1±5.9     |
| Fractional Shortening| %        | 32.3±5.9     | 37.1±4.6     |
| Cardiac Output      | mL/min     | 61.7±3.5     | 64.3±15.9    |
| Renal               |            |              |              |
| RRA-PSV             | mm/s       | 555.9±111.4  | 813.2±447.6  |
| LRA-PSV             | mm/s       | 430.4±149.9  | 490.3±170.5  |
| RRA-LDV             | mm/s       | 143.9±22.5   | 223.6±56.0*  |
| LRA-LDV             | mm/s       | 158.1±65.2   | 191.2±102.9  |
| RRA-RI              | -          | 0.74±0.04    | 0.68±0.13    |
| LRA-RI              | -          | 0.63±0.08    | 0.63±0.09    |
| RRA-PI              | -          | 1.48±0.16    | 1.28±0.46    |
| LRA-PI              | -          | 1.11±0.35    | 1.07±0.34    |

*P < 0.05 versus sham control; statistical evaluations were performed using unpaired t-test. Data in each group are mean ± SEM from four to five rats. RRA-PSV, right renal artery-peak systolic velocity; LRA-PSV, left renal artery-peak systolic velocity; RRA-LDV, right renal artery-lowest diastolic velocity; LRA-LDV, left renal artery-lowest diastolic velocity; RRA-RI, right renal artery-renal arterial resistive index; LRA-RI, left renal artery-renal arterial resistive index; RRA-PI, right renal artery-pulsatility index; LRA-PI, left renal artery-pulsatility index.
checked the gene expression level of group II mGluRs between SHRs and WKYs. In sham control groups, the relative gene expression level of mGluR2 was significantly lower in SHRs (Fig 8A) compared with that in WKYs (Fig 8C). No significant difference in mGluR3 mRNA expression was found between these two strains (Fig 8B and 8D). Moreover, treatment of LY379268 did not affect mRNA expression of mGluR2 and mGluR3 in either rat strains (Fig 8).

Discussion

This study showed that the development of hypertension was weakened by chronic mGluR2/3 agonist treatment in juvenile SHRs. Along with mGluR2/3 agonist treatment, an increase in parasympathetic nervous activity, rather than a decrease in sympathetic nervous activity, was confirmed by the relatively high HF and low LF/HF ratio from the HRV analysis. Therefore, the predominance of parasympathetic nervous activity explains the minimal increase in the BP of SHRs during and after the chronic administration of the mGluR2/3 agonist in the medulla oblongata. This finding was also supported by the lack of changes in blood catecholamine concentration regardless of the agonist treatment. Moreover, echocardiographic data showed no considerable changes in the cardiac function or renal ultrasonographic data, thus failing to support the “non-severe” hypertension effect of the agonist treatment in SHRs. The baroreflex sensitivity was improved with the mGluR2/3 agonist, particularly on reflex bradycardia. In sham control groups, the gene expression of mGluR2 in SHRs was lower than that in WKYs. All these results suggest that the enhancement of parasympathetic nervous activity via mGluR2/3-mediated stimulation may alleviate the development of hypertension and improve baroreflex function.

Although several acute experiments have already attempted to microinject mGluR modulators into the dorsal area of the medulla oblongata including the NTS [7, 13–16], no study had been conducted to date to investigate the chronic stimulation of mGluR2/3 in this brain area. Therefore, the chronic effect of mGluR2/3 stimulation on BP and the toxic effects were preliminary examined to determine the appropriate agonist dose. The results showed that the BP at 1-week post-administration was not substantially different among various concentrations. The best survival rate was observed at 0.4 μg/day, the lowest applied dose. Furthermore, this dose showed potential for chronic application because it was approximately one tenth of the 50% lethal dose (LD_{50} = 4.63 μg/day). Thus, 0.4 μg/day was suggested as the suitable concentration for chronic mGluR2/3 application to suppress the development of hypertension.

Hypertension development is observed in SHRs at 6–12 weeks of age [47, 48]. Given that the 6-week treatment of LY379268 blunted the development of hypertension in young SHRs, mGluR2/3 might be a possible important contributor for patients with essential hypertension. By contrast, SBP was not affected by LY379268 in normotensive rats. This finding suggests that mGluR2/3 is a silent mechanism in normal physiological conditions, especially in blood pressure regulation at the dose used in this study. mGluR involvement for blood pressure regulation has been previously reported, but the specific subtypes of mGluR responsible for this action remain unknown. The diminished SBP response induced by the mGluR agonist was
verified with ACPD, APDC, and 4C3HPG [7, 13, 14, 16]. ACPD is a non-selective mGluR agonist that affects all subtypes of mGluR [13, 16, 49, 50]. 4C3HPG is a weak mGluR2/3 agonist but a potent antagonist of mGluR1 [50, 51]. APDC is a specific mGluR2/3 agonist with lower potency and specificity for mGluR2/3 than LY379268 [50, 51]. The advantage of this study is that the LY379268 treatment was directly administered to the dorsal medulla oblongata; hence, the response of BP and HR might originate directly from NTS, the first cardiovascular site in the dorsal medulla oblongata [5, 6]. The contribution of CVLM and RVLM which can also regulate blood pressure still cannot be excluded [6]. However, cerebrospinal fluid generally moves caudally (e.g., from the fourth ventricles to the cerebellomedullary cistern and then to the central canal) [52] and the NTS has an incomplete blood brain barrier [53], and thus drug effects may be greater at the dorsal side of medulla oblongata rather than at the ventral side where the CVLM and the RVLM located. In addition, the 6-week chronic LY379268 treatment could suppress the onset of hypertension and maintained low hypertensive levels even after its completion. To the authors’ knowledge, this work is the first to study the chronic stimulation of the brainstem, including the NTS, with mGluR2/3 agonists during hypertension development. Although specific and precise targets were not determined for the agonist application, this method might be more clinically relevant than microinjection.

In our study, a slight difference of HR results between the tail-cuff method (Fig 2E) and the telemetry method (Fig 4A) was observed. The HR measurement from the tail-cuff apparatus is the pulse rate under fixation [36]. On the other hand, the HR received from the telemetry device is the results of calculation of R-R interval in unrestrained conditions [40]. The tail-cuff method also required handling for weekly measurements. Therefore, it is considered that the tail-cuff method did not show a decrease in HR throughout the experimental period because of restraint and handling.
The attenuation of hypertensive development is attributed to the following: First, LY379268 treatment could have altered the activity of autonomic nervous function. This notion is based on present and previous findings, in which SHRs had a higher LF level and LF/HF ratio but the same HF level as WKYs [19]. It was considered that LY379268 suppressed the SBP in WKYs depended on the lower LF/HF ratio, which represented the predominance of parasympathetic nerves activity [19, 39]. Second, because catecholamine level was unaffected by LY379268, it seemed that catecholamine was released into the blood without being affected by the parasympathetic dominance found in this study. These results suggested that mGluR2/3 activation in the medulla oblongata might hardly change peripheral resistance, including renal circulation. This finding is in good agreement with the result stating that the high BP level in the hypertensive model could be maintained by increasing vascular responsiveness or sympathetic firing [54]. SHRs have a potential impaired set point for blood pressure regulation with impaired baroreflex function, which may lead to end-organ damage [55, 56]. LY379268 may attenuate hypertensive progression by changing the balance of the sympathetic and parasympathetic nervous systems but not by reforming the set point of blood pressure regulation. Third, SD and CV are often used as indicators of baroreflex sensitivity [57]. Although the change was not throughout the chronical injection period, the increase in SD and CV in SHR

![Fig 7. Effect of LY379268 treatment on baroreflex sensitivity.](https://doi.org/10.1371/journal.pone.0251495.g007)

The attenuation of hypertensive development is attributed to the following: First, LY379268 treatment could have altered the activity of autonomic nervous function. This notion is based on present and previous findings, in which SHRs had a higher LF level and LF/HF ratio but the same HF level as WKYs [19]. It was considered that LY379268 suppressed the SBP in WKYs depended on the lower LF/HF ratio, which represented the predominance of parasympathetic nerves activity [19, 39]. Second, because catecholamine level was unaffected by LY379268, it seemed that catecholamine was released into the blood without being affected by the parasympathetic dominance found in this study. These results suggested that mGluR2/3 activation in the medulla oblongata might hardly change peripheral resistance, including renal circulation. This finding is in good agreement with the result stating that the high BP level in the hypertensive model could be maintained by increasing vascular responsiveness or sympathetic firing [54]. SHRs have a potential impaired set point for blood pressure regulation with impaired baroreflex function, which may lead to end-organ damage [55, 56]. LY379268 may attenuate hypertensive progression by changing the balance of the sympathetic and parasympathetic nervous systems but not by reforming the set point of blood pressure regulation. Third, SD and CV are often used as indicators of baroreflex sensitivity [57]. Although the change was not throughout the chronical injection period, the increase in SD and CV in SHR
after LY379268 administration suggested the possibility of the agonist treatment improving the baroreflex function.

In terms of the baroreflex response, SHRs showed improved reflex bradycardia with LY379268 treatment, but no alteration of reflex tachycardia. LY379268 treatment on SHRs
resulted in parasympathetic dominance, which can stimulate cardiomyocytes through the vagal nerve to release acetylcholine [58]. Then, acetylcholine can bind to their M2 muscarinic receptors to decrease the HR [59]. This reaction, called reflex bradycardia, was observed in the present study. In fact, Rilmenidine, a recognized antihypertensive prescription, reduces sympathetic baroreflex response and increases cardiac vagal baroreflex sensitivity [60]. No differentiation in reflex tachycardia of LY379268-treated SHRs is understandable. Reflex tachycardia usually occurs in hypotension, which is activated during low blood pressure [61]. SHRs already have the risk of developing tachycardia [62]. Although LY379268 did not change the blood’s catecholamine level, it improved baroreflex function as indicated by SD and CV in HRV analysis and improved baroreflex response in reflex bradycardia.

Peripheral vascular resistance and cardiac output can easily manipulate SBP [63]. Cardiac output is mainly associated with HR and pulse pressure, which indicate the difference between SBP and DBP [63, 64]. The LY379268-treated SHRs had lower SBP than the sham control but did not show substantial alterations in their DBP and HR. Cardiac output is an index that is susceptible to changes, but the echocardiographic results in this study showed no effect of LY379268 treatment. Vascular resistance is determined by vascular structure and elastic fibers of its wall. Genetic defects on vascular structure or elastic fibers could induce congenital hypertension, and the critical timing is at fetal and early postnatal stages [63, 65]. Therefore, this mechanism may not be involved in the observed mGluR2/3 treatment effects.

SBP alteration remained in SHRs and WKYs after the treatment, suggesting that blood pressure is maintained by mGluR2/3 per se. The mGluR2/3 at synapses are involved in a series of plasticity processes, including synaptic depression in the NTS [12]. Thus, the synaptic plasticity of mGluR2/3 may contribute to the sustainment of altered blood pressure. On the contrary, mGluR2/3 can also be found on postsynaptic sites or cell bodies in the NTS, and their activation causes neuron membrane hyperpolarization [35]. LY379268 could inhibit the excitability of NTS neurons and thus contribute to hypertension via baroreflex pathways. In this work, the agonist prevented hypertension development to some extent. The neurons responsible for the mechanism might be the GABAergic neurons that project to higher order neurons in the autonomic nervous system.

Greater expression of mGluR2 mRNA was observed in WKYs than SHRs, although no difference in mGluR3 mRNA expression was observed between these two rat strains. Because LY379268 blunted the development of hypertension in SHRs was observed by long-term treatment, more stimulation of fewer receptors may be the effect of maintaining normal blood pressure. It has been reported that mGluR3 desensitized during 7-day administration of LY379268, while mGluR2 did not [66]. If mGluR3 desensitization persisted more than a week in the present study, observed effects were likely to be mGluR2 dependent. However, no effect of long-term LY378268 treatment was observed on the expression of both mGluR2 and mGluR3. Functional and immunohistological studies may be needed to clarify these points. Furthermore, it may also be important to investigate the quantitative and qualitative effects of endogenous agonists.

In severe progression of hypertension on SHRs, hyperactivity of the sympathetic nervous system was observed in the renal region since 2 weeks of age and consequently resulted in significant end-organ damage, including fibrohyalinosis in arterial walls and myocardial hypertrophy with thickened fibers, at the adult age [56, 67]. Given that the cardiac and renal ultrasound examination was performed around 18 weeks of age, end-organ damage was not visible in this study. Although the mechanism underlying the abnormal modulation of the cellular function of hypertensive development remains unclear, mGluR2/3 might have a specific role in blood pressure regulation. Further studies will be needed to clarify the mechanism.
In conclusion, the 6-week chronic stimulation of mGluR2/3 in the dorsal brainstem attenuates the development of hypertension by changing the activity of the autonomic nervous system involving parasympathetic dominance, which also brings better baroreflex function. The crucial mechanism involved in the attenuation of hypertensive development might be the regulation of mGluR2/3 possibly in NTS neurons. These findings may provide novel information for understanding the roles of mGluR2/3 in hypertensive development and potential therapeutic strategies. However, further research is expected to elucidate the underlying cellular mechanisms.

Supporting information

S1 File.

(DOCX)

Acknowledgments

The authors would like to thank Yoshiharu Tsuru, Research Support Department, Primetech Corp., for his technical support with electrocardiography and renal ultrasonography analyses.

Author Contributions

Conceptualization: Julia Chu-Ning Hsu, Shin-ichi Sekizawa, Masayoshi Kuwahara.
Investigation: Julia Chu-Ning Hsu.
Methodology: Julia Chu-Ning Hsu.
Supervision: Masayoshi Kuwahara.
Visualization: Julia Chu-Ning Hsu.
Writing – original draft: Julia Chu-Ning Hsu.
Writing – review & editing: Shin-ichi Sekizawa, Ryota Tochinai, Masayoshi Kuwahara.

References

1. World Health Organization. A global brief on hypertension: Silent killer, global public health crisis. April 2013 [Cited 2020 March 04]. Available from: http://www.who.int/cardiovascular_diseases/publications/global_brief_hypertension/en/.
2. Lamprea-Montalegre JA, Zelnick LR, Hall YN, Bansal N, de Boer IH. Prevalence of hypertension and cardiovascular risk according to blood pressure thresholds used for diagnosis. Hypertension. 2018;72:602–609. https://doi.org/10.1161/HYPERTENSIONAHA.118.11609 PMID: 30354757
3. Fares H, DiNicolantonio JJ, O’Keefe JH, Lavie CJ. Amlodipine in hypertension: A first-line agent with efficacy for improving blood pressure and patient outcomes. Open Heart. 2016;3:e000473. https://doi.org/10.1136/openhrt-2016-000473 PMID: 27792334
4. McCorry LK. Physiology of the autonomic nervous system. Am J Pharm Educ. 2007;71:78. https://doi.org/10.5688/aj710478 PMID: 17786266
5. Talman WT. Glutamatergic transmission in the nucleus tractus solitarii: From server to peripherals in the cardiovascular information superhighway. Braz J Med Biol Res. 1997;30:1–7. https://doi.org/10.1590/s0100-879x1997000100001 PMID: 9222396
6. Colombari E, Sato MA, Cravo SL, Bergamaschi CT, Campos RR Jr, Lopes OU. Role of the medulla oblongata in hypertension. Hypertension. 2001;38:549–554. https://doi.org/10.1161/01.hyp.38.3.549 PMID: 11566929
7. Viard E, Sapru HN. Cardiovascular responses to activation of metabotropic glutamate receptors in the nTS of the rat. Brain Res. 2002;952:308–321. https://doi.org/10.1016/s0006-8993(02)03260-2 PMID: 12376193
8. Blackshaw LA, Page AJ, Young RL. Metabotropic glutamate receptors as novel therapeutic targets on visceral sensory pathways. Front Neurosci. 2011; 5: 40. https://doi.org/10.3389/fnins.2011.00040 PMID: 21472028

9. Anwyl R. Metabotropic glutamate receptors: electrophysiological properties and role in plasticity. Brain Res Brain Res Rev. 1999; 29: 83–120. https://doi.org/10.1016/s0165-0173(98)00050-2 PMID: 9974152

10. Anwyl R. Metabotropic glutamate receptor-dependent long-term potentiation. Neuropharmacology. 2009; 56: 735–740. https://doi.org/10.1016/j.neuropharm.2009.01.002 PMID: 19705571

11. Hay M, McKenzie H, Dietz N, Bradley SR, Conn PJ, et al. Heterogeneity of metabotropic glutamate receptors in autonomic cell groups of the medulla oblongata of the rat. J Comp Neurol. 1999; 403: 486–501. PMID: 9888314

12. Chen CY, Ling Eh, Horowitz JM, Bonham AC. Synaptic transmission in nucleus tractus solitarius is depressed by group II and III but not group I presynaptic metabotropic glutamate receptors in rats. J Physiol. 2002; 538: 773–786. https://doi.org/10.1113/jphysiol.2001.012948 PMID: 11826164

13. Foley CM, Moffitt JA, Hay M, Hasser EM. Glutamate in the nucleus of the solitary tract activates both ionotropic and metabotropic glutamate receptors. Am J Physiol. 1998; 275: R1858–66. https://doi.org/10.1152/ajpregu.1998.275.6.R1858 PMID: 9843874

14. Matsumura K, Tsuchihashi T, Kagiyma S, Abe I, Fujishima M. Subtypes of metabotropic glutamate receptors in the nucleus of the solitary tract of rats. Brain Res. 1999; 842: 461–468. https://doi.org/10.1016/s0006-8993(99)01889-2 PMID: 10526143

15. Antunes VR, Bonagamba LG, Machado BH. NMDA receptor antagonism blocks the cardiovascular responses to microinjection of trans-ACPD into the NTS of awake rats. Exp Physiol. 2004; 89: 279–286. https://doi.org/10.1113/expphysiol.2003.026666 PMID: 15123563

16. Pinto YM, Paul M, Ganten D. Lessons from rat models of hypertension: From Goldblatt to genetic engineering. Cardiovasc Res. 1998; 39: 77–88. https://doi.org/10.1016/s0008-6363(98)00077-7 PMID: 9764191

17. Dickhout JG, Lee RM. Blood pressure and heart rate development in young spontaneously hypertensive rats. Am J Physiol. 1998; 274: H794–H800. https://doi.org/10.1152/ajpheart.1998.274.3.H794 PMID: 9530190

18. Kuwahara M, Hashimoto S, Tsubone H, Sugano S. Developmental changes of autonomic nervous activity in the spontaneously hypertensive rats: Investigation by power spectral analysis of heart rate variability. J Ambul Monit. 1996; 9: 51–58.

19. Chan RK, Chan YS, Wong TM. Electrophysiological properties of neurons in the rostral ventrolateral medulla of normotensive and spontaneously hypertensive rats. Brain Res. 1991; 549: 118–126. https://doi.org/10.1016/0006-8993(91)90607-w PMID: 1893245

20. Matsuura T, Kumaigai H, Kawai A, Onimaru H, Imai M, Oshima N, et al. Rostral ventrolateral medulla neurons of neonatal Wistar-Kyoto and spontaneously hypertensive rats. Hypertension. 2002; 40: 560–565. https://doi.org/10.1161/01.hyp.0000032043.84223.87 PMID: 12364363

21. Dumas TC, Foster TC. Development of metabotropic glutamate receptor-mediated synaptic inhibition. Neuroreport. 1997; 8: 2919–2924. https://doi.org/10.1097/00001756-199709080-00023 PMID: 9376531

22. Jin YH, Bailey TW, Li BY, Schild JH, Andresen MC. Purinergic and vanilloid receptor activation releases glutamate from separate cranial afferent terminals in nucleus tractus solitarius. J Neurosci. 2004; 24: 4709–4717. https://doi.org/10.1523/JNEUROSCI.0753-04.2004 PMID: 15152030

23. Simms AE, Paton JF, Pickering AE. Disinhibition of the cardiac limb of the arterial baroreflex in rat: A role for metabotropic glutamate receptors in the nucleus tractus solitarius. J Physiol. 2006; 575: 727–738. https://doi.org/10.1113/jphysiol.2006.112672 PMID: 16809369

24. Berthele A, Platzer S, Laurie DJ, Weis S, Sommer B, Ziegglansberger W, et al. Expression of metabotropic glutamate receptor subtype mRNA (mGluR1-8) in human cerebellum. Neuroreport. 1999; 10: 3861–3867. https://doi.org/10.1097/00001756-199912160-00026 PMID: 10716224

25. Blanton MG, Lo Turco JJ, Kriegstein AR. Endogenous neurotransmitter activates N-methyl-D-aspartate receptors on differentiating neurons in embryonic cortex. Proc Natl Acad Sci USA. 1990; 87: 8027–8030. https://doi.org/10.1073/pnas.87.20.8027 PMID: 1978317

26. Boer K, Encha-Razavi F, Sinico M, Aronica E. Differential distribution of group I metabotropic glutamate receptors in developing human cortex. Brain Res. 2010; 1324: 24–33. https://doi.org/10.1016/j.brainres.2010.02.005 PMID: 20149785
28. Catania MV, Landwehrmeyer GB, Testa CM, Standaert DG, Penney JB Jr, Young AB. Metabotropic glutamate receptors are differentially regulated during development. Neuroscience. 1994; 61: 481–495. https://doi.org/10.1016/0306-4522(94)90428-6 PMID: 7969925

29. Cho K, Bashir ZI. Cooperation between mglu receptors: A depressing mechanism? Trends Neurosci. 2002; 25: 405–411. https://doi.org/10.1016/S0166-2236(02)02228-2 PMID: 12127757

30. Collingridge GL, Bliss TV. Memories of NMDA receptors and LTP. Trends Neurosci. 1995; 18: 54–56. PMID: 7537406

31. Komuro H, Rakic P. Modulation of neuronal migration by NMDA receptors. Science. 1993; 260: 95–97. https://doi.org/10.1126/science.8096653 PMID: 8096653

32. Mateo Z, Porter JT. Developmental decline in modulation of glutamatergic synapses in layer IV of the barrel cortex by group II metabotropic glutamate receptors. Neuroscience. 2015; 290: 41–48. https://doi.org/10.1016/j.neuroscience.2014.12.083 PMID: 25595699

33. McOmish CE, Pavey G, Gibbons A, Hopper S, Udawela M, Scarr E, et al. Lower [3H]LY341495 binding and the resistive index in renal arteries. Association with long-term progression in chronic renal failure. Nephrol Dial Transplant. 1997; 12: 1376–1380. https://doi.org/10.1093/ndt/12.7.1376 PMID: 9249772

34. Scheetz AJ, Constantine-Paton M. Modulation of NMDA receptor function: Implications for vertebrate neural development. FASEB J. 1994; 8: 745–752. https://doi.org/10.1096/fasebj.8.10.8050674 PMID: 8050674

35. Sekizawa S, Bechtold AG, Tham RC, Bonham AC. A novel postsynaptic group II metabotropic glutamate receptor role in modulating baroreceptor signal transmission. J Neurosci. 2009; 29: 11807–11816. https://doi.org/10.1523/JNEUROSCI.2617-09.2009 PMID: 19776267

36. Kuwahara M, Sugano S, Yayou K, Tsubone H, Kobayashi H. Evaluation of a new tail-cuff method for blood pressure measurements in rats with special reference to the effects of ambient temperature. Exp Anim. 1991; 40: 331–336. https://doi.org/10.1538/expanim1978.40.3_331 PMID: 1915600

37. Tochinai R, Komatsu K, Murakami J, Nagata Y, Ando M, Hata C, et al. Histopathological and functional changes in a single-dose model of combretastatin A4 disodium phosphate-induced myocardial damage in rats. J Toxicol Pathol. 2018; 31: 307–313. https://doi.org/10.1293/tox.2018-0023 PMID: 30393435

38. Petersen LJ, Petersen JR, Tallieruphus U, Ladefoged SD, Mehlsen J, Jensen HA. The pulsatility index and the resistive index in renal arteries. Associations with long-term progression in chronic renal failure. Nephrol Dial Transplant. 1997; 12: 1376–1380. https://doi.org/10.1093/ndt/12.7.1376 PMID: 9249772

39. Kuwahara M, Yayou K, Ishii K, Hashimoto S, Tsubone H, Sugano S. Power spectral analysis of heart rate variability in rats with special reference to the effects of ambient temperature. Exp Anim. 2002; 25: 405–411. https://doi.org/10.1016/s0166-2236(02)02228-2 PMID: 12127757

40. Hashimoto M, Kuwahara M, Tsubone H, Sugano S. Diurnal variation of autonomic nervous activity in the rat: Investigation by power spectral analysis of heart rate variability. J Electrocardiol. 1999; 32: 167–171. PMID: 10338035

41. Akita M, Ishii K, Kuwahara M, Tsubone H. Power spectral analysis of heart rate variability for assessment of diurnal variation of autonomic nervous activity in guinea pigs. Exp Anim. 2002; 51: 1–7. https://doi.org/10.1538/expanim.51.1 PMID: 11871145

42. Burr RL. Interpretation of normalized spectral heart rate variability indices in sleep research: A critical review. Sleep. 2007; 30: 913–919. https://doi.org/10.1093/sleep/30.7.913 PMID: 17682663

43. Almeida J, Oliveira LA, Benini R, Crestani CC. Differential roles of hippocampal nNOS and iNOS in the control of baroreflex function in conscious rats. Brain Res. 2019; 1710: 109–116. https://doi.org/10.1016/j.brainres.2018.12.044 PMID: 30605625

44. Lai CC, Yuan ZF, Chu LY, Chuang KT, Lin HH. Roles of cocaine- and amphetamine-regulated transcript peptide in the rostral ventrolateral medulla in cardiovascular regulation in rats. Brain Res. 2019; 1710: 117–124. https://doi.org/10.1016/j.brainres.2019.01.004 PMID: 30610873

45. Thaeomor A, Wyss JM, Jirakulsomchok D, Roysommuti S. High sugar intake via the renin-angiotensin system blunts the baroreceptor reflex in adult rats that were perinatally depleted of taurine. J Biomed Sci. 2010; 17 Suppl 1: S30. https://doi.org/10.1186/1423-0127-17-S1-S30 PMID: 20804606

46. Crestani CC, Alves FH, Busnardo C, Resstel LB, Correa FM. N-methyl-D-aspartate glutamate receptors in the hypothalamic paraventricular nucleus modulate cardiac component of the baroreflex in unanesthetized rats. Neurosci Res. 2010; 67: 317–326. https://doi.org/10.1016/j.neures.2010.05.001 PMID: 20472007

47. Okamoto K, Aoki K. Development of a strain of spontaneously hypertensive rats. Jpn Circ J. 1963; 27: 282–293. https://doi.org/10.1253/jcj.27.282 PMID: 13939773

48. Anishchenko AM, Aliev OI, Sidekhmenova AV, Shamaanayev AY, Plotnikov MB. Dynamics of Blood Pressure Elevation and Endothelial Dysfunction in SHR Rats During the Development of Articular
Hypertension. Bull Exp Biol Med. 2015; 159: 591–593. https://doi.org/10.1007/s10517-015-3020-8 PMID: 26468032

49. Conn PJ, Pin JP. Pharmacology and functions of metabotropic glutamate receptors. Annu Rev Pharmacol Toxicol. 1997; 37: 205–237. https://doi.org/10.1146/annurev.pharmtox.37.1.205 PMID: 9131252

50. Niswendner CM, Conn PJ. Metabotropic glutamate receptors: Physiology, pharmacology, and disease. Annu Rev Pharmacol Toxicol. 2010; 50: 295–322. https://doi.org/10.1146/annurev.pharmtox.011008.145533 PMID: 20055706

51. Flor PJ, Battaglia G, Nicoletti F, Gasparini F, Bruno V. Neuroprotective activity of metabotropic glutamate receptor ligands. Adv Exp Med Biol. 2002; 513: 197–223. https://doi.org/10.1007/978-1-4615-0123-7_7 PMID: 12575822

52. Yamada S, Miyazaki M, Kanazawa H, Higashi M, Morohoshi Y, Bluml S, et al. Visualization of cerebrospinal fluid movement with spin labeling at MR imaging: Preliminary results in normal and pathophysiological conditions. Radiology. 2008; 249: 644–652. https://doi.org/10.1148/radiol.249207185 PMID: 18936318

53. Gross PM, Wall KM, Pang JJ, Shaver SW, Wainman DS. Microvascular specializations promoting rapid interstitial solute dispersion in nucleus tractus solitarius. Am J Physiol. 1990; 259: R1131–R1138. https://doi.org/10.1152/ajpregu.1990.259.6.R1131 PMID: 2260724

54. Julius S, Nesbitt S. Sympathetic overactivity in hypertension: A moving target. Am J Hypertens. 1996; 9: 113S–120S. https://doi.org/10.1016/0895-7061(96)00287-7 PMID: 8931844

55. Takahashi H, Yoshika M, Komiyama Y, Nishimura M. The central mechanism underlying hypertension: A review of the roles of sodium ions, epithelial sodium channels, the renin-angiotensin-aldosterone system, oxidative stress and endogenous digitalis in the brain. Hypertens Res. 2011; 34: 1147–1160. https://doi.org/10.1038/hr.2011.105 PMID: 21814209

56. Shan ZZ, Dai SM, Su DF. Relationship between baroreceptor reflex function and end-organ damage in spontaneously hypertensive rats. Am J Physiol. 1999; 277: H1200–H1206. https://doi.org/10.1152/ajpheart.00724.2007 PMID: 18055517

57. Chang CC, Hsiao TC, Chiang YY, Hsu HY. The usefulness of the coefficient of variation of electrocardiographic RR interval as an index of cardiovascular function and its correlation with age and stroke. Tungs’ Med J. 2013; 6: 41–48.

58. Manabe N, Foldes FF, Tőrőcsik A, Nagashima H, Goldiner PL, Vizi ES. Presynaptic interaction between vagal and sympathetic innervation in the heart: Modulation of acetylcholine and noradrenaline release. J Auton Nerv Syst. 1991; 32: 233–242. https://doi.org/10.1016/0165-1838(91)90117-l PMID: 1645381

59. LaCroix C, Freeling J, Giles A, Wess J, Li YF. Deficiency of M2 muscarinic acetylcholine receptors increases susceptibility of ventricular function to chronic adrenergic stress. Am J Physiol Heart Circ Physiol. 2008; 294: H810–H820. https://doi.org/10.1152/ajpheart.00724.2007 PMID: 18055517

60. Head GA. Baroreflexes and cardiovascular regulation in hypertension. J Cardiovasc Pharmacol. 1995; 26 Suppl 2: S7–S16. PMID: 8642810

61. Dünser MW, Hasibeder WR. Sympathetic overstimulation during critical illness: Adverse effects of adrenergic stress. J Intensive Care Med. 2009; 24: 293–316. https://doi.org/10.1177/0885066094304191 PMID: 19703817

62. Palatini P, Casiglia E, Pauletto P, Staessen J, Kaciroti N, Julius S. Relationship of tachycardia with high blood pressure and metabolic abnormalities: A study with mixture analysis in three populations. Hypertension. 1997; 30: 1267–1273. https://doi.org/10.1161/01.hyp.30.5.1267 PMID: 9369286

63. Mayet J, Hughes A. Cardiac and vascular pathophysiology in hypertension. Heart. 2003; 89: 1104–1109. https://doi.org/10.1136/heart.89.9.1104 PMID: 12923045

64. Koenig J, Hill LK, Williams DP, Thayer JF. Estimating cardiac output from blood pressure and heart rate: The liljestrand & zander formula. Biomed Sci Instrum. 2015; 51: 85–90. PMID: 25996703

65. Grisk O, Rettig R. Interactions between the sympathetic nervous system and the kidneys in arterial hypertension. Cardiovasc Res. 2004; 61: 238–246. https://doi.org/10.1016/j.cardiores.2003.11.024 PMID: 14736540