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# Even weak vasoconstriction from rilmenidine can be unmasked *in vivo* by opening the baroreflex feedback loop

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ABSTRACT

*Aims:* Rilmenidine and moxonidine are centrally acting antihypertensive agents that are more selective for $I_1$-imidazoline receptors than for $\alpha_2$-adrenergic receptors. Moxonidine previously showed a peripheral vasoconstrictive effect stronger than generally recognized, which counteracted an arterial pressure (AP) lowering effect resulting from central sympathoinhibition. We tested whether rilmenidine also showed a significant vasoconstrictive effect that could attenuate its AP lowering effect.

*Main methods:* Efferent sympathetic nerve activity (SNA) and AP responses to changes in carotid sinus pressure were compared in nine anesthetized Wistar–Kyoto rats before and after low, medium, and high doses (40, 100, and 250 μg/kg, respectively) of intravenous rilmenidine.

*Key findings:* High-dose rilmenidine narrowed the range of the SNA response (from 89.6 ± 2.9% to 50.4 ± 7.9%, $P < 0.001$) and reduced the lower asymptote of SNA (from 13.5 ± 3.0% to 2.7 ± 1.5%, $P < 0.001$). High-dose rilmenidine significantly increased the intercept (from 57.1 ± 3.8 to 78.2 ± 2.7 mm Hg, $P < 0.001$) but reduced the slope (from 0.82 ± 0.08 to 0.51 ± 0.07 mm Hg/%, $P < 0.001$) of the SNA–AP relationship. The reduction in the operating-point AP induced by high-dose rilmenidine did not significantly differ based on whether the peripheral effect was considered (−19.8 ± 2.2 *vs.* −26.4 ± 5.3 mm Hg, not significant).

*Significance:* Rilmenidine increased AP in the absence of SNA, which suggests a peripheral vasoconstrictive effect; however, the vasoconstrictive effect was weak and did not significantly counteract the AP-lowering effect through central sympathoinhibition.

## 1. Introduction

Rilmenidine and moxonidine are centrally acting antihypertensive agents that act mainly on $I_1$-imidazoline receptors. Although these agents are structural analogs of clonidine, there are some structural differences — moxonidine retains an imidazoline ring, whereas rilmenidine has an oxazoline ring [1]. It has been postulated that $I_1$-imidazoline receptors are located upstream from $\alpha_2$-adrenergic receptors within the neural pathway at the rostral ventrolateral medulla (RVLM), and that activation of $I_1$-imidazoline receptors, through activation of $\alpha_2$-adrenergic receptors, reduces sympathetic outflow [2]. It is possible, however, that the effect of a given sympatholytic drug on arterial pressure (AP) is not determined by only its central action when the drug is administered systemically. In a previous study, we systematically analyzed the effect of intravenously administered moxonidine on the sympathetic regulation of AP using an open-loop systems analysis [3]. In that study, the carotid sinus baroreflex was divided into two principal reflex arcs — a neural arc from carotid sinus pressure (CSP) to efferent sympathetic nerve activity (SNA) and a peripheral arc from SNA to AP [4,5]. Intravenous moxonidine moved the peripheral arc upward; *i.e.*, it increased AP at any given level of SNA, which suggests moxonidine-induced vasoconstriction. The AP-lowering effect of moxonidine through central sympathoinhibition is significantly attenuated by peripheral vasoconstriction [3]. Nevertheless, the peripheral vasoconstrictive effect of moxonidine has not attracted wide attention because central sympathoinhibition overrides peripheral vasoconstriction and induces vasodilation as a net effect.

With regard to the central effect, moxonidine has three times more

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affinity to $I_1$-imidazoline receptors than rilmenidine in the bovine RVLM membrane, although the two agents show a comparable affinity toward $I_1$-imidazoline receptors relative to $\alpha_2$-adrenergic receptors (the affinity ratio of approximately 30) [6]. Hence, moxonidine might be more effective than rilmenidine in suppressing SNA at the same dose range. Conversely, with regard to the peripheral effect, rilmenidine does not show a significant vasoconstrictive effect on the rat tail artery [7]. The lack of a significant vasoconstrictive effect in the *in vitro* study suggests that rilmenidine might be more suitable than moxonidine as an antihypertensive drug. Meanwhile, an *in vivo* study using a pithed-rabbit preparation demonstrated a direct vasoconstrictive effect of rilmenidine on the renal artery in the absence of renal SNA [8]. Whole-body experiments are necessary for a comprehensive assessment of the central and peripheral effects of rilmenidine on sympathetic AP regulation.

A caveat often associated with whole-body experiments is that changes in SNA affect AP through the cardiovascular system, whereas changes in AP affect SNA through the baroreflex neural arc [5]. The closed-loop negative-feedback operation of the arterial baroreflex makes it difficult to separately assess the central and peripheral effects of a given drug. The baroreflex negative-feedback loop must be opened to assess the significance of the central and peripheral effects relative to each other. We hypothesized that rilmenidine would exert some peripheral vasoconstriction that could offset the AP-lowering effect resulting from central sympathoinhibition.

## 2. Materials and methods

The experimental animals were cared for in strict accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences, which has been approved by the Physiological Society of Japan. The Animal Subjects Committee at the National Cerebral and Cardiovascular Center reviewed and approved the experimental protocols.

### 2.1. Surgical procedures

The experiment was performed on male Wistar–Kyoto (WKY) rats (350–420 g, 397 ± 20 g, mean ± SD). The rat was anesthetized by intraperitoneal injection of an anesthetic mixture (2 mL/kg) containing 40 mg/mL urethane and 250 mg/mL α-chloralose. The rat was mechanically ventilated with oxygen-enriched air. The anesthetic mixture was diluted 18-fold with physiological saline and continuously administered (2–3 $mL{\cdot}kg^{-1}{\cdot}h^{-1}$) from a catheter inserted into the right femoral vein. Another catheter was inserted into the left femoral vein for administering rilmenidine or vehicle. AP was measured from a catheter inserted into the right femoral artery. Heart rate (HR) was measured from a body-surface electrocardiogram.

The postganglionic branch of the left splanchnic sympathetic nerve was exposed through a left flank incision. A pair of stainless steel wire electrodes (AS633, Cooner Wire, Chatsworth, CA, USA) was attached to the nerve and secured using silicone glue (Kwik-Sil, World Precision Instruments, Sarasota, FL, USA). The nerve signal was preamplified and band-pass filtered between 150 and 1000 Hz, then full-wave rectified and low-pass filtered using a cutoff frequency of 30 Hz to quantify SNA. Hexamethonium bromide (60 mg/kg) was intravenously injected at the end of the experiment to block ganglionic transmission and determine the noise level of the SNA recording. After that, high-dose pentobarbital (200 mg/kg) was intravenously administered to euthanize the animal.

The bilateral carotid sinus baroreceptor regions were isolated from systemic circulation [9,10]. The isolated baroreceptor regions were cannulated from the common carotid arteries, and intracarotid sinus pressure (CSP) was controlled using a servo-controlled piston pump system. The bilateral vagal and aortic depressor nerves were sectioned at the neck to minimize any confounding reflex effects from the cardiopulmonary regions and aortic arch.

### 2.2. Protocol

Rilmenidine protocol ($n$ = 9): Rilmenidine hemifumarate (Funakoshi, Tokyo, Japan) was dissolved in dimethyl sulfoxide (DMSO) (Wako Pure Chemical Industries, Osaka, Japan) and diluted with physiological saline to a 1-mg/mL solution (1% v/v DMSO). The solution was further diluted with physiological saline to 40-, 100-, and 250-μg/mL rilmenidine solutions (0.04, 0.1, and 0.25% v/v DMSO), which were used for low-dose (40 μg/kg), medium-dose (100 μg/kg), and high-dose (250 μg/kg) administrations, respectively. Each rilmenidine solution was administered at 1 mL/kg cumulatively as described in the next paragraph. Although the final cumulative dose of rilmenidine was 390 μg/kg, an earlier study indicated that increasing the dose of intravenous rilmenidine from 300 to 1000 μg/kg can further reduce AP in spontaneously hypertensive rats (SHR) [11]. Hence, the notation of "high dose" needs to be interpreted as a relative dose within this paper. When we tested 1000-μg/kg rilmenidine after the high-dose rilmenidine in one rat (the final cumulative dose of 1390 μg/kg), SNA was reduced close to the noise level irrespective of the CSP level, and the neural arc could not be analyzed by assuming a typical sigmoidal input–output relationship.

To estimate the open-loop static characteristics of the carotid sinus baroreflex over the entire input pressure range, CSP was first decreased to 60 mm Hg for 5 min and then increased in a stepwise manner up to 180 mm Hg in increments of 20 mm Hg. Each incremental step lasted 1 min. The CSP input sequence was repeated and designated as S1 through S9. The baroreflex responses were observed before administering rilmenidine (S1 and S2). One minute after completing S2, low-dose rilmenidine was intravenously administered, and its effect was evaluated during S4. One minute after completing S4, medium-dose rilmenidine was intravenously added, and its effect was evaluated during S6. One minute after completing S6, high-dose rilmenidine was intravenously added, and its effect was evaluated during S8.

DMSO protocol ($n$ = 5): DMSO has several hemodynamic effects [12]. To confirm the lack of a significant effect of DMSO used as a solvent, DMSO was diluted by physiological saline to a 1% v/v DMSO solution and intravenously administered instead of the rilmenidine solutions. The DMSO solution was administered three times at 1 mL/kg each (10 μL/kg of DMSO) one minute after completing S2, S4, and S6. This protocol also served as a time control.

### 2.3. Data analyses

Data were recorded at 1000 Hz using an analog-to-digital converter [AIO AD16-16(PCI)EV, Contec, Japan]. Values of SNA, AP, and HR were averaged for the last 10 s of each CSP step. The relationships between CSP and SNA (the neural arc), CSP and AP (the total reflex arc), and CSP and HR were quantified by fitting the following four-parameter logistic function to the data [13]:

$$y = \frac{P_1}{1 + exp[P_2(CSP - P_3)]} + P_4$$

where $y$ is the output (SNA, AP, or HR), $P_1$ is the response range, $P_2$ is the slope coefficient, $P_3$ is the midpoint of the sigmoid curve on the CSP axis, and $P_4$ is the lower asymptote of the sigmoid curve. The maximum gain, $G_{max}$, is calculated from $P_1P_2/4$.

A linear regression analysis was used to quantify the relationship between SNA and AP (the peripheral arc) [3,5,14] as follows:

$$AP = b_0 + b_1 SNA$$

where $b_0$ and $b_1$ are the intercept and slope of the regression line, respectively.

A baroreflex equilibrium diagram was obtained by plotting the fitted neural and peripheral arcs on a pressure–SNA plane [15,16]. The intersection between the neural and peripheral arcs provides the operating point at which SNA and AP settle when the baroreflex negative-

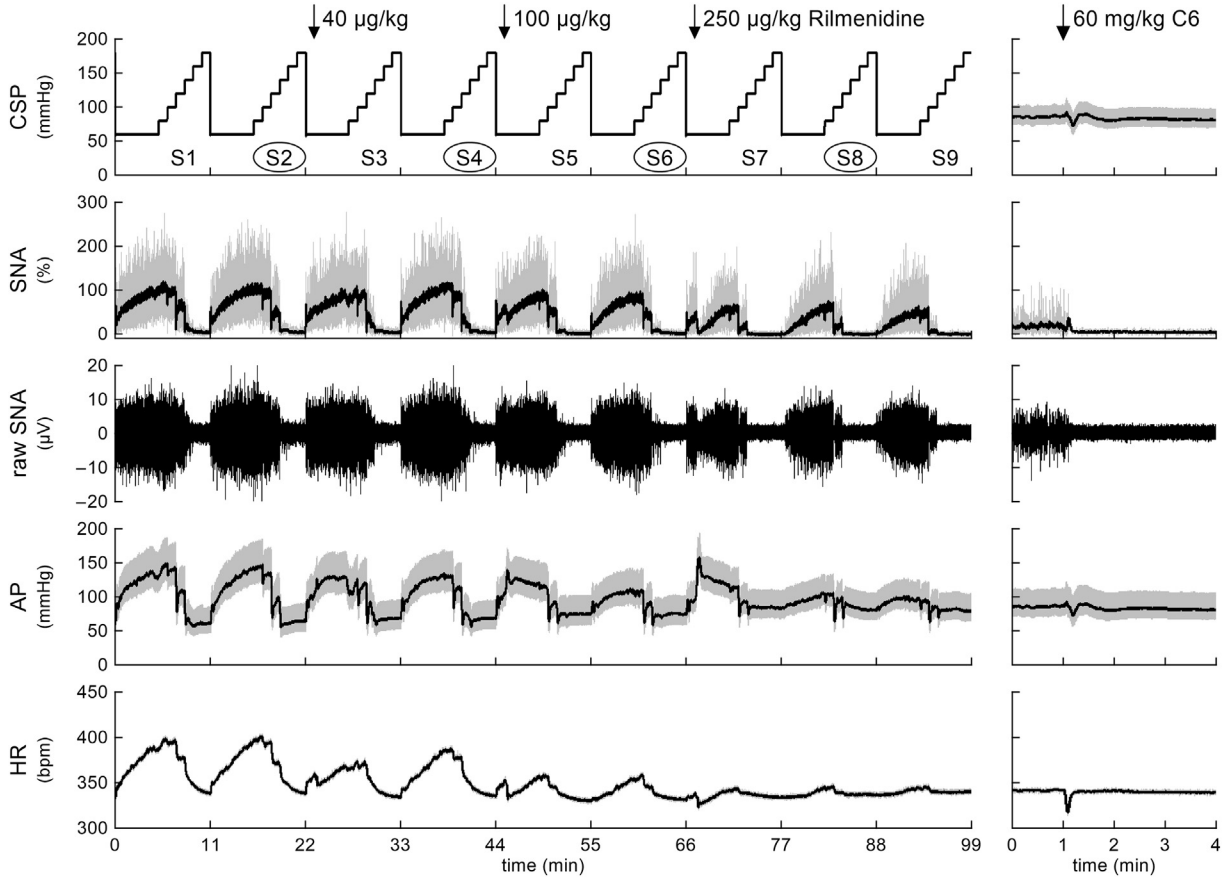


**Fig. 1.** Typical time series of carotid sinus pressure (CSP), sympathetic nerve activity (SNA), raw SNA, arterial pressure (AP), and heart rate (HR). CSP is plotted as a 10-Hz resampled signal. The gray and black lines in the SNA plot indicate 10-Hz resampled and 2-s moving averaged signals, respectively. The raw SNA is plotted using the data of an original sampling frequency (1000 Hz). The gray and black lines in the AP and HR plots represent 200-Hz resampled and 2-s moving averaged signals, respectively (the 2-s moving averaged signal is nearly superimposed on the 200-Hz resampled signal in the HR plot). In the left panels, CSP was changed in a stepwise manner, and the input sequences were designated as S1 through S9. In each sequence, an increase in CSP decreased SNA, AP, and HR. Low-dose (40 μg/kg), medium-dose (100 μg/kg), and high-dose (250 μg/kg) rilmenidine were intravenously administered 1 min after completing S2, S4, and S6 sequences, respectively. The baroreflex responses were compared among S2, S4, S6, and S8 sequences. In the right panels, an intravenous administration of hexamethonium bromide (C6) eliminated the burst activity in SNA. bpm, beats/min.

feedback loop is closed.

### 2.4. Statistical analyses

The parameters of the baroreflex static characteristics were compared among conditions of control, low-dose, medium-dose, and high-dose rilmenidine. One-way repeated-measures analysis of variance (ANOVA) followed by Tukey's test was used for simultaneous multiple comparisons (Prism, GraphPad, La Jolla CA, USA). A paired *t*-test was used to compare a reduction of the operating-point AP caused by each dose of rilmenidine between conditions with or without considering the peripheral effect. The differences were considered significant at $P < 0.05$. The data obtained from the DMSO protocol were likewise analyzed among conditions of control and the first, second, and third DMSO administrations.

## 3. Results

A representative time series obtained from the rilmenidine protocol in one rat is shown in Fig. 1. In the left panels, the stepwise increase in CSP decreased SNA, AP, and HR. The cardiac-locked activity of SNA was not observed even when the time scale was expanded because of the nonpulsatile nature of CSP. The baroreflex responses observed in sequence S2 were treated as the control. One minute after completing S2, low-dose rilmenidine was administered, which did not significantly affect the SNA response but decreased the maximum AP and HR in response to low CSP input during S4. One minute after completing S4, medium-dose rilmenidine was added, which transiently increased AP. The maximum SNA, AP, and HR in response to low CSP input were lower during S6 than during S4. One minute after completing S6, high-dose rilmenidine was added, which transiently decreased SNA but increased AP. The maximum SNA, AP, and HR in response to low CSP input were lower during S8 than during S6. In the right panels, hexamethonium reduced SNA to the noise level.

Fig. 2 summarizes the effects of rilmenidine on the open-loop baroreflex characteristics. In the total reflex arc (Fig. 2A), one-way repeated-measures ANOVA indicated that the overall effects of rilmenidine were significant on the response range, $P_1$, the midpoint pressure, $P_3$, the lower asymptote, $P_4$, and the maximum gain, $G_{max}$. The *post-hoc* analysis indicated that low-dose rilmenidine did not significantly affect the parameters. Rilmenidine administered at the medium and high doses significantly decreased $P_1$, $P_3$, and $G_{max}$ but increased $P_4$. In the HR control (Fig. 2B), the overall effects of rilmenidine were significant on all parameters except $P_2$. Rilmenidine administered at the medium and high doses significantly decreased $P_1$, $P_3$, $P_4$, and $G_{max}$. In the neural arc (Fig. 2C), the overall effects of rilmenidine were significant on all parameters except $P_2$. Low-dose rilmenidine significantly decreased $P_3$, medium-dose rilmenidine significantly decreased $P_3$ and $P_4$, and high-dose rilmenidine significantly decreased $P_1$, $P_3$, $P_4$, and $G_{max}$. In the peripheral arc (Fig. 2D), the overall effects of rilmenidine were

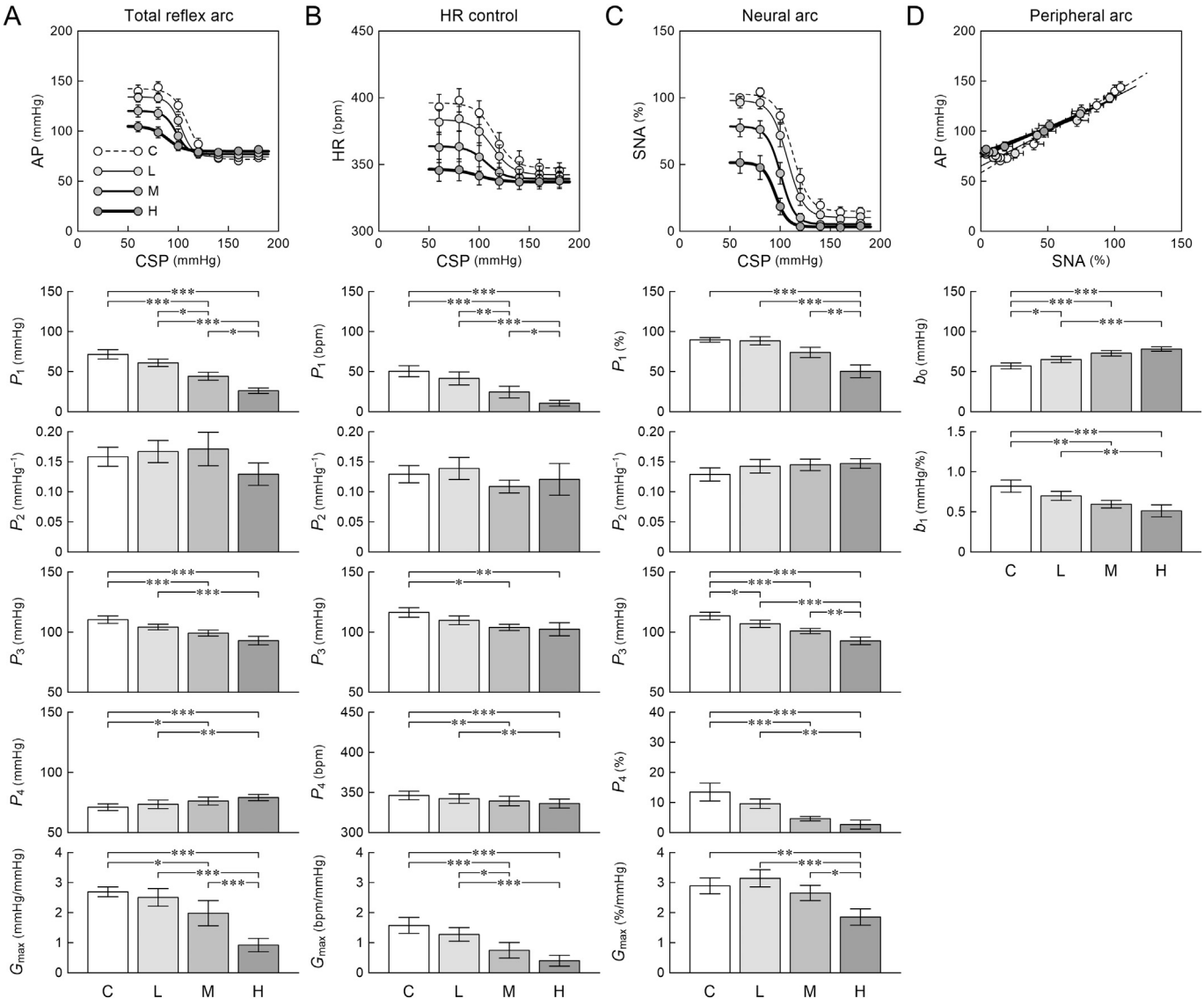


**Fig. 2.** Open-loop static characteristics of the total reflex arc (A), heart rate (HR) control (B), neural arc (C), and peripheral arc (D) and their parameter values obtained from the rilmenidine protocol. Data were obtained under control conditions (C) and after administering low-dose (L), medium-dose (M), and high-dose (H) rilmenidine. AP, arterial pressure; CSP, carotid sinus pressure; SNA, sympathetic nerve activity; bpm, beats/min; $P_1$ through $P_4$, parameters of the fitted sigmoid curve ($P_1$, response range; $P_2$, slope coefficient; $P_3$, midpoint input pressure; $P_4$, lower asymptote); $G_{max}$, maximum gain; $b_0$ and $b_1$, intercept and slope of the linear regression. One-way repeated-measures analysis of variance (ANOVA) followed by Tukey's test was used for statistical analyses. Data are the means ± SE ($n$ = 9). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

significant on the intercept, $b_0$, and the slope, $b_1$. Rilmenidine significantly increased $b_0$ in a dose-dependent manner, and significantly decreased $b_1$ at the medium and high doses.

The baroreflex equilibrium diagrams were drawn by plotting the neural and peripheral arcs on a pressure-SNA plane (Fig. 3A). The intersection of the neural and peripheral arcs gives the operating point. The overall effects of rilmenidine on the operating-point AP, SNA, and gain were significant. Rilmenidine at the medium and high doses significantly decreased the operating-point AP, SNA, and gain. The baroreflex equilibrium diagrams were also constructed without considering the peripheral effect of rilmenidine (Fig. 3B). When the reduction from the control value was calculated, the removal of the peripheral effect did not significantly affect the magnitude of the reduction in the operating-point AP induced by low-, medium-, or high-dose rilmenidine (Fig. 3C).

Fig. 4 summarizes the effects of intravenous injections of DMSO (10 μL/kg) on the open-loop baroreflex characteristics. Except for a decreasing trend in the lower asymptote of the HR response to CSP, administrations of the DMSO solution did not significantly affect the total reflex arc, neural arc, or peripheral arc characteristics.

## 4. Discussion

The present study has demonstrated that rilmenidine increased the intercept of the peripheral arc in a dose-dependent manner (Fig. 2D), which suggests a vasoconstrictive effect of rilmenidine. The vasoconstrictive effect, however, was small relative to the central sympathoinhibitory effect such that removing the peripheral effect did not significantly change the magnitude of the reduction in the operating-point AP at any dose of rilmenidine (Fig. 3C).

### 4.1. Peripheral effect of intravenous rilmenidine

Rilmenidine and moxonidine are centrally acting antihypertensive agents that reduce sympathetic outflow from the central nervous system [2]. In our previous study [3], intravenous moxonidine (100-μg/kg

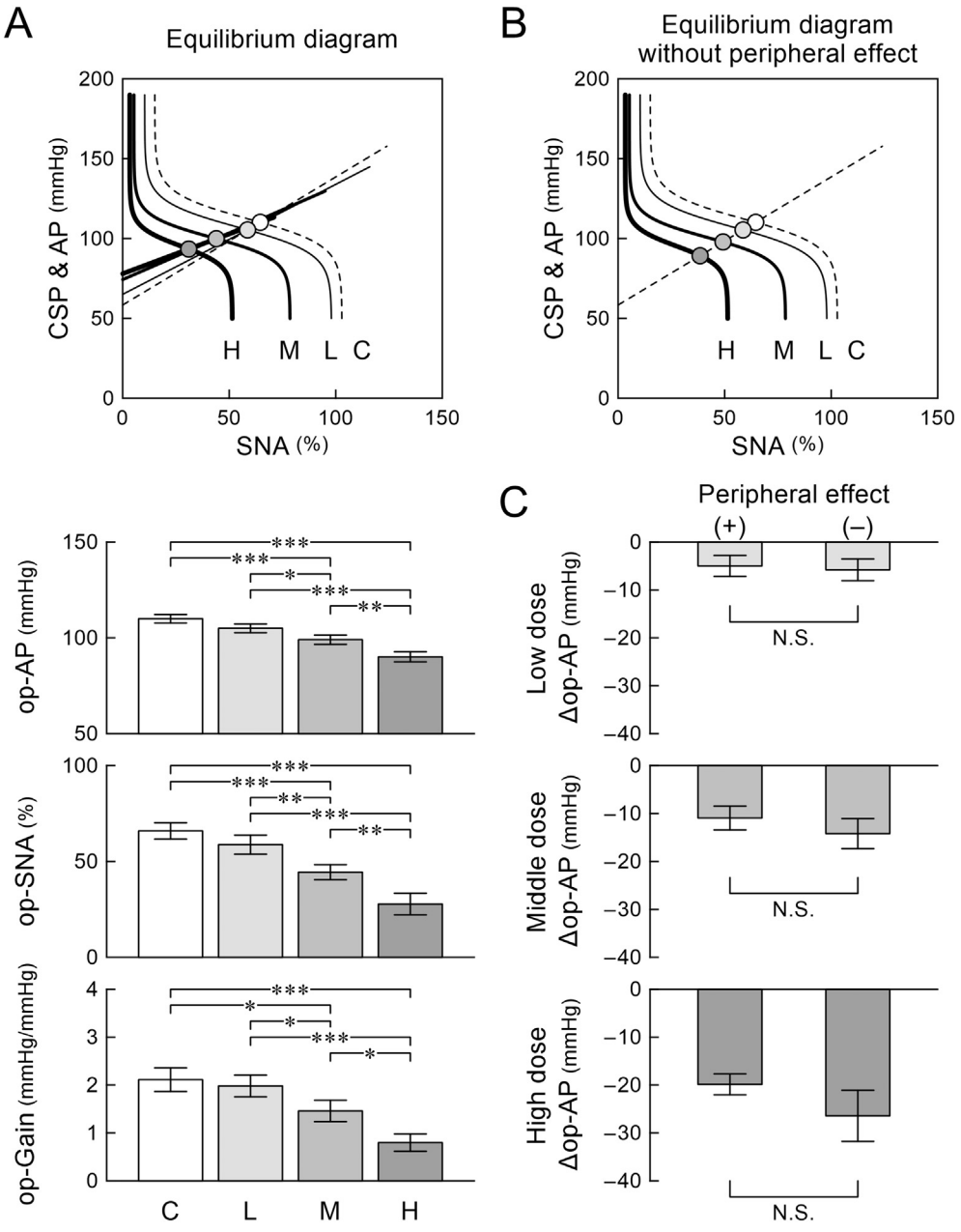


**Fig. 3.** Baroreflex equilibrium diagrams obtained by plotting mean neural and peripheral arcs on a pressure–sympathetic nerve activity (SNA) plane and bar graphs showing operating-point arterial pressure (op-AP), operating-point SNA (op-SNA), and operating-point gain (op-Gain) estimated from equilibrium diagrams of individual animals (A). Data were obtained under control conditions (C) and after administering low-dose (L), medium-dose (M), and high-dose (H) rilmenidine. Effects of rilmenidine on op-AP, op-SNA, and op-Gain were tested using one-way repeated-measures analysis of variance (ANOVA) followed by Tukey's test. Baroreflex equilibrium diagrams can be constructed without changing the peripheral arc from that under control conditions (B). The reductions in op-AP from the control value (Δop-AP) were compared between the baroreflex equilibrium diagrams with (+) or without (−) considering the peripheral effect of rilmenidine using a paired *t*-test (C). CSP, carotid sinus pressure; AP, arterial pressure. Data are the means ± SE (n = 9). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and N.S., not significant.

bolus injection followed by a continuous infusion at 200 μg·kg$^{-1}$·h$^{-1}$) exerts a vasoconstrictive effect that is stronger than that generally recognized. The vasoconstrictive effect of moxonidine significantly counteracts the centrally-mediated AP-lowering effect. In that study, the baroreflex equilibrium diagram predicted that the reduction in the operating-point AP caused by moxonidine would be augmented from −8.8 ± 1.5 to −31.9 ± 5.7 mm Hg ($P < 0.01$, $n = 8$) if not for the peripheral effect. A similar argument can be applied to an $\alpha_{2A}$-adrenergic agonist guanfacine. Although intravenously administered guanfacine decreases SNA at any given CSP level, the peripheral vasoconstrictive effect nearly cancels the reduction in the operating-point AP [14]. On reanalyzing the experimental data on guanfacine, we suggest that the removal of the peripheral effect would augment a reduction in the operating-point AP from −5.3 ± 2.7 to −10.5 ± 2.4 mm Hg ($P < 0.05$, $n = 5$) at low-dose guanfacine (20 μg/kg) and from −5.6 ± 5.1 to −25.0 ± 5.9 mm Hg ($P < 0.01$, $n = 7$) at high-dose guanfacine (100 μg/kg). These results indicate that peripheral vasoconstriction can significantly modify the AP-lowering effect caused by a given central antihypertensive agent.

In the present study, the intercept of the peripheral arc represents AP in the absence of sympathetic tone. High-dose rilmenidine increased the intercept by approximately 20 mm Hg (Fig. 2D), which indicates a direct vasoconstrictive effect. Although we used DMSO as a solvent, DMSO (1% v/v) alone did not significantly affect the peripheral arc characteristics (Fig. 4D). The result of the increased intercept might be in line with a vasoconstrictive effect of rilmenidine on the renal artery observed in the absence of renal SNA [8]. The increase in the intercept, however, did not significantly affect the operating-point AP, as demonstrated in the baroreflex equilibrium diagram (Fig. 3). This was because high-dose rilmenidine decreased the slope of the peripheral arc to approximately 62% of the control value (from 0.82 ± 0.08 to 0.51 ± 0.07 mm Hg/%) despite increasing the intercept. In our previous study, moxonidine also reduced the slope of the peripheral arc to approximately 72%, but the increase in the intercept was so large (~50 mm Hg) that the reduction in the slope could not effectively cancel the effect of the increased intercept on the operating-point AP [3]. Hence, rilmenidine has a weaker vasoconstrictive effect than moxonidine in relation to the reduction in the operating-point AP. As

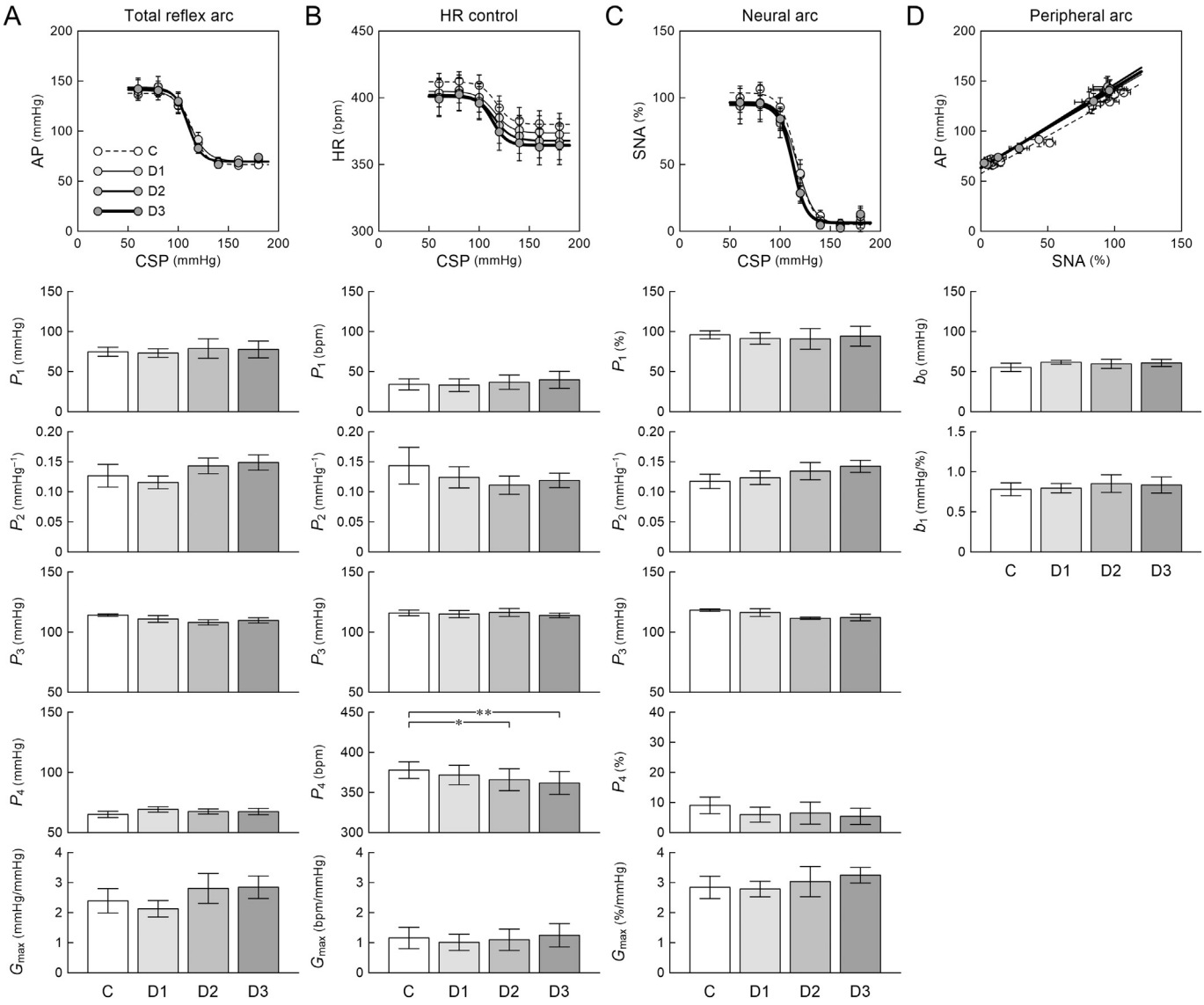


**Fig. 4.** Open-loop static characteristics of the total reflex arc (A), heart rate (HR) control (B), neural arc (C), and peripheral arc (D) and their parameter values obtained from the dimethyl sulfoxide (DMSO) protocol. Data were obtained under control conditions (C) and after administering the first (D1), second (D2), and third (D3) injections of DMSO (10 μL/kg each). AP, arterial pressure; CSP, carotid sinus pressure; SNA, sympathetic nerve activity; bpm, beats/min; $P_1$ through $P_4$, parameters of the fitted sigmoid curve ($P_1$, response range; $P_2$, slope coefficient; $P_3$, midpoint input pressure; $P_4$, lower asymptote); $G_{max}$, maximum gain; $b_0$ and $b_1$, intercept and slope of the linear regression. One-way repeated-measures analysis of variance (ANOVA) followed by Tukey's test was used for statistical analyses. Data are the means ± SE ($n = 5$). $^{*}P < 0.05$ and $^{**}P < 0.01$.

for the mechanisms by which the slope of the peripheral arc is reduced, rilmenidine might inhibit the release of norepinephrine from sympathetic nerve terminals by acting on presynaptic $\alpha_2$-adrenergic receptors [17]. Moxonidine might reduce the release of norepinephrine by acting on $I_1$-binding sites in addition to $\alpha_2$-adrenergic receptors on the sympathetic neurons [18].

### 4.2. Central effect of intravenous rilmenidine

Clonidine is a first-generation centrally acting antihypertensive agent that acts on both $I_1$-imidazoline and $\alpha_2$-adrenergic receptors to suppress sympathetic outflow from the central nervous system. The stimulation of $\alpha_2$-adrenergic receptors in the treatment of hypertension is associated with unwanted side effects such as sedation and dry mouth [1]. Second-generation centrally acting antihypertensive agents such as rilmenidine and moxonidine are highly selective for $I_1$-imidazoline receptors and reduce the side effects related to stimulation of $\alpha_2$-adrenergic receptors. Rilmenidine has exhibited a better tolerance profile than clonidine and α-methyldopa with regard to side effects mediated by α-adrenergic receptors [19]. Nevertheless, it has been shown that expression of the $\alpha_{2A}$-adrenergic receptors is a prerequisite for the cardiovascular effects of moxonidine and rilmenidine [20]. The current understanding is that $I_1$-imidazoline receptors are located upstream from $\alpha_2$-adrenergic receptors in the RVLM and exert a sympathoinhibitory effect by activating those $\alpha_2$-adrenergic receptors [2]. On the other hand, sedation might be associated with $\alpha_2$-adrenergic stimulation at the locus coeruleus [21], where $I_1$-imidazoline receptor agonists have little effect [22].

In the present study, rilmenidine significantly reduced the response range and the lower asymptote of the neural arc (Fig. 2C), which confirms the central sympathoinhibitory effect. Rilmenidine also decreased the midpoint input pressure of the CSP–SNA relationship in a dose-dependent manner. These changes are consistent with the effect of rilmenidine injection into the RVLM on the mean AP–renal SNA relationship estimated in a conscious rabbit preparation [2]. The central effect was not due to DMSO used as a solvent (Fig. 4C). The arterial

baroreflex function is known to be modified over time, which is called baroreceptor resetting [23]. Although the baroreceptor resetting can take place even in acute experimental conditions and its application to pharmacotherapy is suggested [24], the DMSO protocol did not show a significant time-dependent change in the neural or peripheral arc. Maintaining CSP at 60 mm Hg might have reset the baroreflex toward the lower input pressure, and maintaining CSP at 180 mm Hg might have reset the baroreflex toward the higher input pressure; nevertheless, the effect of the resetting was not identified as a time effect, probably due to the repeated nature of the stepwise CSP input (*i.e.*, the resetting also repeatedly occurred without producing significant time-dependent changes across the CSP input sequences). This does not mean, however, that the stepwise CSP input protocol is not suitable for detecting all types of baroreceptor resetting. As an example, the neural arc of SHR showed a significant resetting toward the higher input pressure compared with that of WKY [25].

Since the vagal and aortic depressor nerves were sectioned, the SNA response mainly resulted from changes in CSP. It should be noted, however, that afferent vagal stimulation resets the baroreflex neural arc and inhibits SNA [26], and vagotomy increases SNA at a given CSP in Sprague-Dawley rats [27]. These results suggest the presence of a tonic inhibitory effect on SNA mediated by the vagal nerves, which may be partly attributable to low-pressure baroreflex activation [28]. Hence, the vagotomy might have increased baseline SNA and made the effect of rilmenidine on SNA more easily detectable compared with conditions of intact vagi. As for the aortic baroreflex, the aortic depressor nerves terminate centrally to the nucleus tractus solitarius (NTS) as do the carotid sinus nerves, and the two baroreflex systems share common neural pathways in the regulation of the cardiovascular system [29]. Since the neural pathways from the carotid sinus and aortic baroreflexes converge on the RVLM, which is the major site of sympathoinhibitory action by rilmenidine, the effect of rilmenidine on the neural arc may be qualitatively similar between conditions with and without the aortic denervation. However, if we estimate the carotid sinus baroreflex open-loop characteristics without the aortic denervation, the aortic baroreflex counteracts the carotid sinus baroreflex and attenuates the baroreflex responses [30].

### 4.3. Effects of intravenous rilmenidine on total reflex arc and HR control

Rilmenidine significantly reduced the response range of the total reflex arc with a slight increase in the lower asymptote (Fig. 2A). Hence, rilmenidine decreased AP only when baseline AP was high. Rilmenidine also decreased the maximum gain of the total reflex arc (Fig. 2A), which is attributable to reductions in both the maximum gain of the neural arc (Fig. 2C) and the slope of the peripheral arc (Fig. 2D). A reduction in total reflex gain can reduce stability of AP against pressure disturbances, which can lead to postural hypotension; however, no indication of postural hypotension was reported during rilmenidine treatment in clinical trials on elderly patients [19]. The increase in the lower asymptote of the total reflex arc, resulting from the peripheral vasoconstrictive effect of rilmenidine, could help avoid hypotensive events.

Rilmenidine reduced the response range of HR with a slight decrease in the lower asymptote and decreased the midpoint input pressure of the CSP–HR relationship (Fig. 2B). Rilmenidine does not show a direct chronotropic effect in an isolated rat atria preparation [31]. Because the vagal nerves were sectioned in the present study, changes in the CSP–HR relationship should be nearly parallel with those in the neural arc (CSP–SNA relationship), although a possible regional difference between the cardiac and splanchnic SNAs might affect the HR response. The time effect might have also partly contributed to the decrease in the lower asymptote of the HR response because the lower asymptote also decreased in the DMSO protocol (Fig. 4B). The reduction in the response range and the maximum gain observed in the HR control are consistent with the effects on the mean AP–HR relationship of rilmenidine injection into the fourth ventricle after vagal nerve blockade (intravenous methscopolamine) in a conscious rabbit preparation [32].

### 4.4. Limitations

First, the acute effect of rilmenidine on the carotid sinus baroreflex was examined in anesthetized rats. Hence, the results cannot be directly applied to the chronic effect of rilmenidine on conscious subjects. Second, the effect of rilmenidine on vagal outflow was not examined because of the vagotomy. Rilmenidine can facilitate cardiac vagal response [2], and further studies on animals with intact vagi are necessary to elucidate the entire effects of the drug on autonomic cardiovascular regulation. Third, the effect of rilmenidine could be different in hypertensive animals. As an example, SHR exhibits higher SNA than WKY [25]; and thus, the central effect of rilmenidine could be more pronounced in SHR. Studies on animal models of hypertension are also necessary to clarify this matter.

## 5. Conclusions

Rilmenidine increased the intercept of the peripheral arc of the carotid sinus baroreflex, which suggests a vasoconstrictive effect; however, the vasoconstrictive effect did not significantly affect the reduction in the operating-point AP. These characteristics are different from those of moxonidine or guanfacine, which demonstrate significant vasoconstrictive effects that counteract the AP-lowering effect from central sympathoinhibition. Separate assessment of drug effects on the neural and peripheral arcs can more fully elucidate the differential characteristics of antihypertensive agents with regard to determining the operating-point AP.

## Conflict of interest

The authors declare that there are no conflicts of interest.

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