

Published in final edited form as:

Hypertension. 2013 August ; 62(2): 255–262. doi:10.1161/HYPERTENSIONAHA.113.01466.

Regulation of Hypothalamic Presympathetic Neurons and Sympathetic Outflow by Group II Metabotropic Glutamate Receptors in Spontaneously Hypertensive Rats

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Abstract

Increased glutamatergic input in the hypothalamic paraventricular nucleus (PVN) plays an important role in the development of hypertension. Group II metabotropic glutamate receptors (mGluRs) are expressed in the PVN, but their involvement in regulating synaptic transmission and sympathetic outflow in hypertension is unclear. Here we show that the group II mGluRs agonist (2S,2'R,3'R)-2-(2',3'-dicarboxycyclopropyl)glycine (DCG-IV) produced a significantly greater reduction in the frequency of spontaneous and miniature excitatory postsynaptic currents and the amplitude of electrically evoked excitatory postsynaptic currents in retrogradely labeled spinally projecting PVN neurons in spontaneously hypertensive rats (SHRs) than in normotensive control rats. DCG-IV similarly decreased the frequency of GABAergic inhibitory postsynaptic currents of labeled PVN neurons in the two groups of rats. Strikingly, DCG-IV suppressed the firing of labeled PVN neurons only in SHRs. DCG-IV failed to inhibit the firing of PVN neurons of SHRs in the presence of ionotropic glutamate receptor antagonists. Lowering blood pressure with celiac ganglionectomy in SHRs normalized the DCG-IV effect on excitatory postsynaptic currents to the same level seen in control rats. Furthermore, microinjection of DCG-IV into the PVN significantly reduced blood pressure and sympathetic nerve activity in SHRs. Our findings provide new information that presynaptic group II mGluR activity at the glutamatergic terminals increases in the PVN in SHRs. Activation of group II mGluRs in the PVN inhibits sympathetic vasomotor tone through attenuation of increased glutamatergic input and neuronal hyperactivity in SHRs.

Keywords

hypertension; hypothalamus; sympathetic nervous activity; synaptic transmission; glutamate receptors

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Conflict(s) of Interest/Disclosure(s)

None.

Introduction

The paraventricular nucleus (PVN) of the hypothalamus contributes to the development of hypertension^{1–3}. A population of PVN neurons projects to preganglionic sympathetic neurons in the spinal cord and rostral ventrolateral medulla and is likely involved in regulating sympathetic outflow and blood pressure^{4–7}. Elevated sympathetic nerve activity has been shown in patients with essential hypertension^{8–10}, and the hyperactivity of PVN presympathetic neurons has been demonstrated in animal models of essential hypertension, such as spontaneously hypertensive rats (SHRs)^{11–15}. However, the precise mechanisms underlying hyperactivity of PVN presympathetic neurons in hypertension are not fully known.

Glutamate is the major excitatory neurotransmitter in the PVN, and increased glutamatergic input into PVN presympathetic neurons leads to elevated sympathetic vasomotor tone in SHRs^{3,12}. In addition to fast-acting ionotropic glutamate receptors, G protein–coupled metabotropic glutamate receptors (mGluRs) are also activated by glutamate. Eight different mGluRs have been cloned and classified into three groups¹⁶. Group I mGluRs (mGluR1 and mGluR5) are coupled to $G_{q/11}$ proteins to activate phospholipase C and protein kinase C, which increase neuronal firing activity and synaptic transmission. In contrast, group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6, mGluR7, and mGluR8) mGluRs are coupled to $G_{i/o}$ proteins, which reduce neuronal excitability and synaptic transmission¹⁷. Although mGluR2/3 are widely distributed in the brain, including the PVN^{18,19} and median preoptic nucleus²⁰, the functional significance of group II mGluRs in the PVN in the control of glutamatergic input and sympathetic vasomotor tone in hypertension has not been determined.

In the present study, we tested the hypothesis that group II mGluRs control the excitability of PVN presympathetic neurons in hypertension by regulating glutamatergic input. In addition, we determined the role of group II mGluRs in the PVN in the regulation of sympathetic outflow in hypertension. Our findings provide new information that the activity of group II mGluRs at the glutamatergic terminals in the PVN increases in hypertension, which may provide a mechanism to dampen excessive glutamate release. Activation of group II mGluRs restrains the excitability of PVN presympathetic neurons and sympathetic vasomotor tone in hypertension.

Methods

Male Wistar-Kyoto (WKY) rats and SHRs (13 weeks old; Harlan, Indianapolis, IN) were used in this study. We used 89 SHRs and 79 WKY rats for the entire study. The surgical procedures and experimental protocols were approved by the Institutional Animal Care and Use Committee and conformed to the National Institutes of Health guidelines for the ethical use of animals. The arterial blood pressure (ABP) of the rats was measured every day for 1 week before the final experiments, using a non-invasive tail-cuff system. The systolic ABP was significantly higher in SHRs (208 ± 3.01 mmHg, $n = 25$) than in age-matched WKY rats (122.18 ± 2.56 mmHg, $n = 25$).

The detailed methods for retrograde labeling of spinally projecting PVN neurons, electrophysiological recordings of glutamatergic excitatory postsynaptic currents (EPSCs) and inhibitory postsynaptic currents (IPSCs) in brain slices, PVN microinjections and lumbar sympathetic nerve recording *in vivo*, and data analysis are described in Online Supplements.

Results

Group II mGluR activity is increased to regulate glutamate release to PVN presympathetic neurons in hypertension

To determine the role of group II mGluRs in controlling glutamatergic input to PVN presympathetic neurons in hypertension, we examined the effect of DCG-IV, a highly specific group II mGluR agonist^{21,22}, on spontaneous EPSCs (sEPSCs) of spinally projecting PVN neurons in SHR and WKY rats. The mean basal frequency of sEPSCs in labeled PVN neurons was significantly higher in SHR than in WKY rats (Fig. 1, A,B). Bath application of DCG-IV (0.1, 0.3, 1.0, and 3.0 μ M; each applied for about 4 min) significantly decreased the frequency, but not the amplitude, of sEPSCs in a concentration-dependent manner in both WKY rats and SHR (Fig. 1, A–C). Notably, DCG-IV caused a significantly greater reduction in the frequency of sEPSCs in SHR than in WKY rats (Fig. 1C). Bath application of 100 nM LY341495, a highly selective group II mGluR antagonist^{22,23}, had no significant effect on the basal frequency of sEPSCs. LY341495 abolished the inhibitory effect of DCG-IV (1 μ M) on the frequency of sEPSCs in labeled PVN neurons in both WKY rats and SHR (Fig. 1D).

To further determine the activity of presynaptic group II mGluRs at glutamatergic terminals in hypertension, we examined the effect of DCG-IV on mEPSCs of spinally projecting PVN neurons in SHR and WKY rats. Bath application of 1 μ M DCG-IV significantly inhibited the frequency, but not the amplitude, of mEPSCs in labeled PVN neurons in both WKY rats and SHR (Fig. 2, A–D). The cumulative probability analysis of mEPSCs revealed that the distribution pattern of the inter-event interval of mEPSCs was shifted toward the right in response to DCG-IV (Fig. 2, B,C). The inhibition of the frequency of mEPSCs by DCG-IV in labeled PVN neurons was significantly greater in SHR than in WKY rats (Fig. 2C).

We next confirmed the presynaptic effects of DCG-IV on evoked EPSCs by including a general G protein inhibitor, GDP- β -s (1 mM), in the pipette solution to block the postsynaptic effect of DCG-IV²². In WKY rats, bath application of 1 μ M DCG-IV significantly decreased the amplitude of evoked EPSCs in the labeled PVN neurons (44.09 \pm 4.23% reduction, n = 8). In SHR, DCG-IV caused a much larger reduction in the amplitude of evoked EPSCs (60.74 \pm 5.68% reduction, n = 8, P < 0.05; Fig. 3A). Bath application of 100 nM LY341495 abolished the inhibitory effects of DCG-IV on evoked EPSCs of PVN neurons in both WKY rats and SHR (Fig. 3B). In addition, bath application of 1 μ M DCG-IV had no significant effect on the amplitude of currents elicited by puff AMPA (100 μ M) application onto labeled PVN neurons in WKY rats and SHR (Fig. 3C).

It has been shown that group II mGluRs are present on glia in some brain regions²⁴. To determine the possible contribution of these group II mGluRs located on glia to the inhibitory effect of DCG-IV on glutamatergic input to PVN neurons in SHR, we treated brain slices with fluoroacetate, a selective inhibitor of glial metabolism²⁵. Incubating brain slices with 5 mM fluoroacetate for 30–50 min did not significantly change the inhibitory effect of DCG-IV on evoked EPSCs of PVN neurons in SHR (Fig. 3D). Collectively, these results strongly suggest that the activity of presynaptic group II mGluRs at glutamatergic terminals is increased in the PVN in hypertension.

Increased presynaptic group II mGluR activity in the PVN in hypertension is an adaptive response to increased ABP

To determine whether the increased group II mGluR activity is the cause of hypertension or a secondary adaptive response to increased ABP, we examined the effects of DCG-IV on evoked EPSCs (recorded with GDP- β -s included in the pipette solution) of spinally projecting PVN neurons in SHR two weeks after lowering the ABP with CGx¹⁴.

Compared with the sham surgery, CGx significantly lowered the mean ABP, measured with radiotelemetry, for at least 2 weeks in SHR rats (Fig. 3E). CGx had no effects on the food or water intake and body weight in all rats studied. DCG-IV (1 μ M) produced a significantly smaller inhibitory effect on evoked EPSCs in labeled PVN neurons in SHR rats undergoing CGx than in the sham group (Fig. 3F). Notably, the effect of DCG-IV on evoked EPSCs of labeled PVN neurons in SHR rats subjected to CGx was not significantly different from that in WKY rats.

Group II mGluR activity controlling GABAergic input to PVN presympathetic neurons does not change in hypertension

The activity of PVN presympathetic neurons is finely tuned by both excitatory glutamatergic and inhibitory GABAergic inputs^{12,26,27}. To determine whether the control of GABAergic input by the activity of group II mGluRs in the PVN is altered in hypertension, we compared the effect of DCG-IV on GABAergic IPSCs of spinally projecting PVN neurons in WKY rats and SHR rats. The basal frequency of spontaneous IPSCs (sIPSCs) was significantly lower in the PVN in SHR rats than in WKY rats (Fig. 4, A,B), and the amplitude of sIPSCs was significantly smaller in SHR rats than in WKY rats (Fig. 4, A,B). Bath application of DCG-IV (1 μ M) significantly decreased the frequency, but not the amplitude, of sIPSCs in both WKY rats and SHR rats (Fig. 4, A,B). The inhibitory effect of DCG-IV on the frequency of sIPSCs did not differ significantly between WKY rats and SHR rats (Fig. 4B).

The basal frequency and amplitude of mIPSCs in labeled PVN neurons were also significantly reduced in SHR rats compared with WKY rats (Fig. 4C). Bath application of 1 μ M DCG-IV significantly decreased the frequency, but not the amplitude, of mIPSCs in both WKY rats and SHR rats. The inhibitory effect of DCG-IV on the frequency of mIPSCs in labeled PVN neurons did not differ significantly between WKY rats and SHR rats (Fig. 4C).

We also examined the effect of DCG-IV on evoked IPSCs (recorded with GDP- β -s included in the pipette solution) of spinally projecting PVN neurons in WKY rats and SHR rats. The basal amplitude of evoked IPSCs was significantly smaller in SHR rats than in WKY rats (Fig. 4, D,E). DCG-IV (1 μ M) produced a similar magnitude of reduction in the amplitude of evoked IPSCs in WKY rats and SHR rats (Fig. 4E). These data suggest that the activity of presynaptic group II mGluRs at GABAergic terminals in the PVN is not altered in hypertension.

Group II mGluRs regulate the firing activity of PVN presympathetic neurons in hypertension

To determine the role of group II mGluRs in the control of the firing of PVN presympathetic neurons in hypertension, we tested the effect of DCG-IV on the firing activity of labeled PVN neurons with cell-attached recordings in SHR rats and WKY rats. The basal firing rate of PVN neurons in SHR rats was significantly higher than that in WKY rats. Bath application of DCG-IV (1 μ M) had no significant effect on the firing activity of PVN neurons in WKY rats. In contrast, DCG-IV significantly decreased the firing rate of PVN neurons in SHR rats (Fig. 5, A,B). The group II mGluR antagonist LY341495 abolished the inhibitory effect of DCG-IV on the firing activity in SHR rats (Fig. 5C).

In additional labeled PVN neurons in SHR rats, blocking NMDA and AMPA receptors with their respective antagonists, AP-5 (50 μ M) and CNQX (20 μ M), significantly reduced their basal firing activity (Fig. 5D). In the presence of AP5 and CNQX, DCG-IV failed to further inhibit the firing activity of these PVN neurons in SHR rats (Fig. 5D). These results suggest that activation of group II mGluRs inhibits the firing of PVN presympathetic neurons through attenuation of glutamatergic input in hypertension.

Group II mGluRs in the PVN control sympathetic vasomotor tone in hypertension

To determine the role of group II mGluRs in the PVN in controlling sympathetic outflow in hypertension, we microinjected DCG-IV directly into the PVN. Bilateral microinjection of DCG-IV (10–20 pmol/50 nl) slightly increased LSNA and mean ABP in WKY rats ($n = 7$ rats; Fig. 6, A,B). In contrast, in SHR, microinjection of DCG-IV (5–20 pmol/50 nl) into the PVN significantly decreased LSNA, mean ABP, and HR in a dose-dependent manner ($n = 7$ rats). Bilateral microinjection of LY341495 (6 pmol/50 nl) had no significant effect on LSNA, mean ABP, and HR in WKY rats or SHR (Fig. 6C). LY341495 abolished the effect of DCG-IV on LSNA, mean ABP, and HR in SHR and WKY rats (Fig. 6C). These data suggest that activation of group II mGluRs in the PVN inhibits sympathetic outflow in hypertension.

During the course of this study, 2 WKY rats and 3 SHR were excluded from the data analysis because of the misplacement of microinjections (Fig. 6D). Microinjection of DCG-IV (20 pmol/50 nl) had minimal effect on ABP (WKY: 85.8 ± 4.6 vs. 84.3 ± 3.7 mmHg; SHR: 138.5 ± 8.5 vs. 140.1 ± 6.2 mmHg), LSNA (WKY: 100% vs. $101.5 \pm 0.5\%$; SHR: 100% vs. $101.4 \pm 1.7\%$), and HR (WKY: 278.5 ± 10.5 vs. 282.0 ± 9.0 bpm; SHR: 340.8 ± 12.1 vs. 344.8 ± 12.3 bpm) in these 2 WKY rats and 3 SHR.

Discussion

The major objective of our study was to determine the role of group II mGluRs in the control of synaptic input to PVN presympathetic neurons and sympathetic outflow in a commonly used animal model of essential hypertension. At the present time, there are no available methods to unequivocally identify PVN neurons involved only in ABP control in brain slices. We acknowledge that not all spinally projecting PVN neurons are exclusively involved in ABP regulation. Group II mGluRs are located at presynaptic terminals, postsynaptic neurons, and glia in several brain regions²⁸. In this study, we found that activation of group II mGluRs with DCG-IV significantly inhibited the frequency, but not the amplitude, of sEPSCs and mEPSCs in spinally projecting PVN neurons in both WKY rats and SHR. DCG-IV also significantly reduced the amplitude of electrically evoked EPSCs when GDP- β -s was intracellularly dialyzed to inhibit the postsynaptic G proteins. Furthermore, DCG-IV had no significant effect on postsynaptic AMPA receptor currents elicited by puff AMPA application. In addition, we found that the inhibitory effect of DCG-IV on evoked EPSCs of PVN neurons was not altered brain slices were treated with fluoroacetate to inhibit glial function, which suggests that glial cells are not involved in the control of glutamatergic input by group II mGluRs in the PVN. Our findings indicate that activation of group II mGluRs inhibits glutamatergic input to PVN presympathetic neurons through a presynaptic mechanism.

An important finding of our study is that the activity of group II mGluRs on glutamatergic terminals is increased in the PVN in hypertension. This conclusion is supported by our data showing that the effects of DCG-IV on the frequency of sEPSCs and mEPSCs and the amplitude of evoked EPSCs in the PVN were significantly greater in SHR than in WKY rats. Interestingly, lowering ABP in SHR with CGx normalized the inhibitory effect of DCG-IV on evoked EPSCs of PVN neurons to the same level observed in WKY rats. This finding suggests that the increased activity of group II mGluRs at the presynaptic glutamatergic terminals in the PVN in SHR is a secondary adaptive response to increased ABP. Although it is not clear whether this increased activity of presynaptic group II mGluRs in SHR results from an increased receptor number or from signal coupling, the increased activity of group II mGluRs at the glutamatergic terminals may constrain synaptic glutamate release to PVN presympathetic neurons in hypertension.

The firing activity of PVN presympathetic neurons is finely tuned by both glutamatergic and GABAergic inputs^{3,26,27,29}. We have shown that the inhibitory GABAergic input to PVN presympathetic neurons is diminished in SHR^{11,15,30}. In the present study, we found that DCG-IV significantly reduced the amplitude of evoked IPSCs of spinally projecting PVN neurons in SHR and WKY rats. DCG-IV treatment also significantly inhibited the frequency, but not the amplitude, of GABAergic sIPSCs and mIPSCs of PVN neurons in both groups. These data suggest that the activation of group II mGluRs also inhibits GABAergic transmission through a presynaptic mechanism. However, the degree of presynaptic effects of DCG-IV in the PVN did not differ significantly between SHR and WKY rats. These findings suggest that the activity of group II mGluRs at GABAergic terminals is not altered in the PVN in hypertension.

The increase in excitatory glutamatergic input to PVN presympathetic neurons in SHR is caused by augmentation of pre- and postsynaptic NMDA receptor activity by the protein kinase CK2^{12,31}. Interestingly, we found that DCG-IV inhibited the firing activity of spinally projecting PVN neurons only in SHR, although DCG-IV inhibited synaptic glutamate release in both SHR and WKY rats. The lack of an inhibitory effect of DCG-IV on the firing of PVN neurons in WKY rats is probably due to the fact that group II mGluR activation also removed inhibitory GABAergic input to PVN neurons in WKY rats. Because of augmented excitatory glutamatergic input and diminished inhibitory GABAergic input in the PVN in SHR^{3,11,12,30}, activation of group II mGluRs could reduce the excitability of PVN presympathetic neurons by attenuation of increased glutamatergic input in hypertension. This hypothesis is supported by our finding that the inhibitory effect of DCG-IV on the firing activity of PVN neurons in SHR was abolished when NMDA and AMPA receptors were blocked. Although the presynaptic glutamatergic neuronal soma may not be present within the PVN, group II mGluRs present on the presynaptic terminals are directly involved in controlling the firing activity of postsynaptic (spinally projecting) neurons in the PVN in SHR.

Another significant finding of our study is that activation of group II mGluRs in the PVN inhibited sympathetic vasomotor tone only in SHR. Microinjection of DCG-IV into the PVN induced a significant and dose-dependent reduction in the ABP and LSNA in SHR. These results are consistent with our brain slice recordings showing an inhibitory effect of DCG-IV on the firing activity of PVN neurons only in SHR. However, we unexpectedly found that microinjection of DCG-IV into the PVN slightly increased the ABP and LSNA in WKY rats at high doses. It is not clear how DCG-IV in the PVN increases ABP and sympathetic nerve activity in WKY rats. It is unlikely that DCG-IV produces a non-specific effect, because blocking group II mGluRs with LY341495 abolished the DCG-IV effect in both SHR and WKY rats. Because GABAergic inhibition of PVN neuronal excitability is more pronounced in WKY rats than in SHR^{11,15,30}, removal of GABAergic input by the group II mGluR agonist may disinhibit PVN presympathetic neurons *in vivo*, leading to a small increase in sympathetic outflow in WKY rats. Although we did not observe a stimulatory effect of DCG-IV on neuronal firing in brain slices of WKY rats, it is possible that certain inhibitory interneurons might have been removed in our thin brain slice preparation.

Perspectives

We found that activation of group II mGluRs inhibits both glutamatergic and GABAergic transmission at a presynaptic site in the PVN. The activity of group II mGluRs at glutamatergic terminals is increased in hypertension, which may limit excessive glutamate release through a negative feedback mechanism. This finding provides further evidence of synaptic and neuronal plasticity in the PVN associated with hypertension. We also show that

activation of group II mGluRs inhibits the hyperactivity of PVN presympathetic neurons and sympathetic vasomotor tone only in SHRs and not in normotensive rats. On the basis of these findings, group II mGluRs may represent a potential therapeutic target for the treatment of neurogenic hypertension. It should be noted that because the group II mGluR agonist was microinjected into the PVN, it can inhibit sympathetic outflow by affecting neurons other than just spinally projecting neurons. Further studies are also needed to determine changes in presynaptic group II mGluRs in the PVN in other models of hypertension and to examine the long-term effect of the group II mGluR agonist in conscious animal models of hypertension.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Source(s) of Funding

This study was supported by the National Institutes of Health (grant HL077400), a postdoctoral fellowship from the American Heart Association–South Central Affiliate (to Z.-Y.Y), and the N.G. and Helen T. Hawkins Endowment (to H.-L.P.).

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Novelty and Significance

1. What Is New:
 - Presynaptic group II mGluRs regulate excitatory and inhibitory synaptic input to presympathetic neurons in the hypothalamus
 - Group II mGluR stimulation inhibits hyperactivity of hypothalamic presympathetic neurons in hypertension by reducing glutamatergic input
 - Group II mGluR activation in the hypothalamus reduces sympathetic nerve discharges and arterial blood pressure in hypertension
2. What Is Relevant:
 - Increased glutamatergic input and excitability of presympathetic neurons in the hypothalamus contribute to elevated sympathetic output in hypertension
 - Group II mGluRs may be targeted for treatment of hypertension by reducing the sympathetic vasomotor tone.
3. Summary:
 - Activation of group II mGluRs in the hypothalamus inhibits sympathetic drive and hyperactivity of presympathetic neurons by reducing synaptic glutamate release.

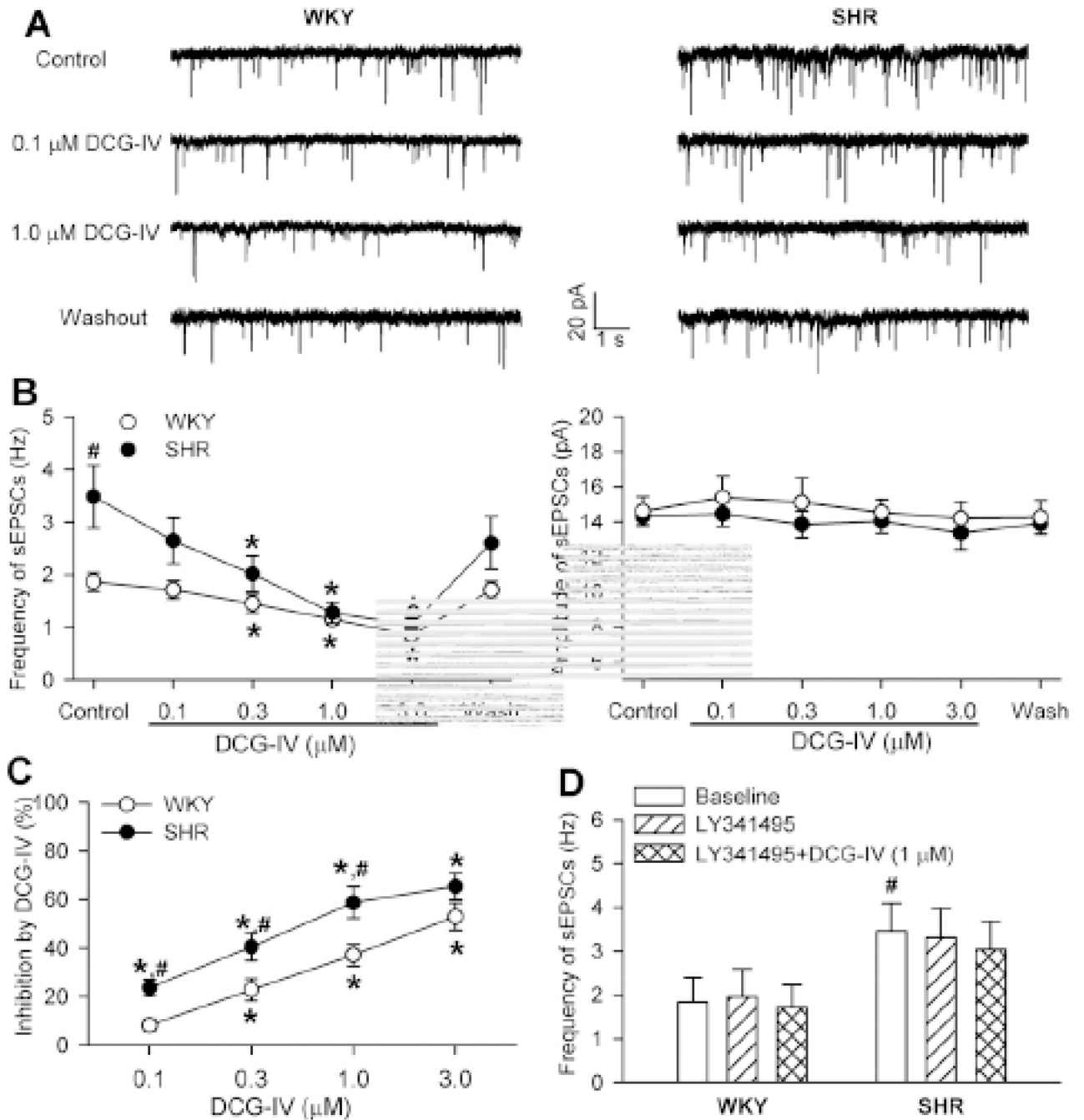


Figure 1. DCG-IV inhibition of sEPSCs of spinal projecting PVN neurons is greater in SHR than in WKY rats

A, Representative traces show sEPSCs recorded from labeled PVN neurons during control and application of DCG-IV (0.1 and 1 μM) in 1 SHR and 1 WKY rat. B, Summary data show the concentration-dependent inhibitory effect of DCG-IV on the frequency and amplitude of sEPSCs in WKY rats (n = 8) and SHRs (n = 7). C, Comparison of the inhibitory effect of DCG-IV on the frequency of sEPSCs of PVN neurons (shown in B) in WKY rats and SHRs. D, Group data show that LY341495 (100 nM) blocked the effect of DCG-IV (1 μM) on the frequency of sEPSCs of labeled PVN neurons in SHRs and WKY

rats (n = 7 neurons in each group). * P < 0.05, compared with the respective baseline control. # P < 0.05, compared with the corresponding value in WKY rats.

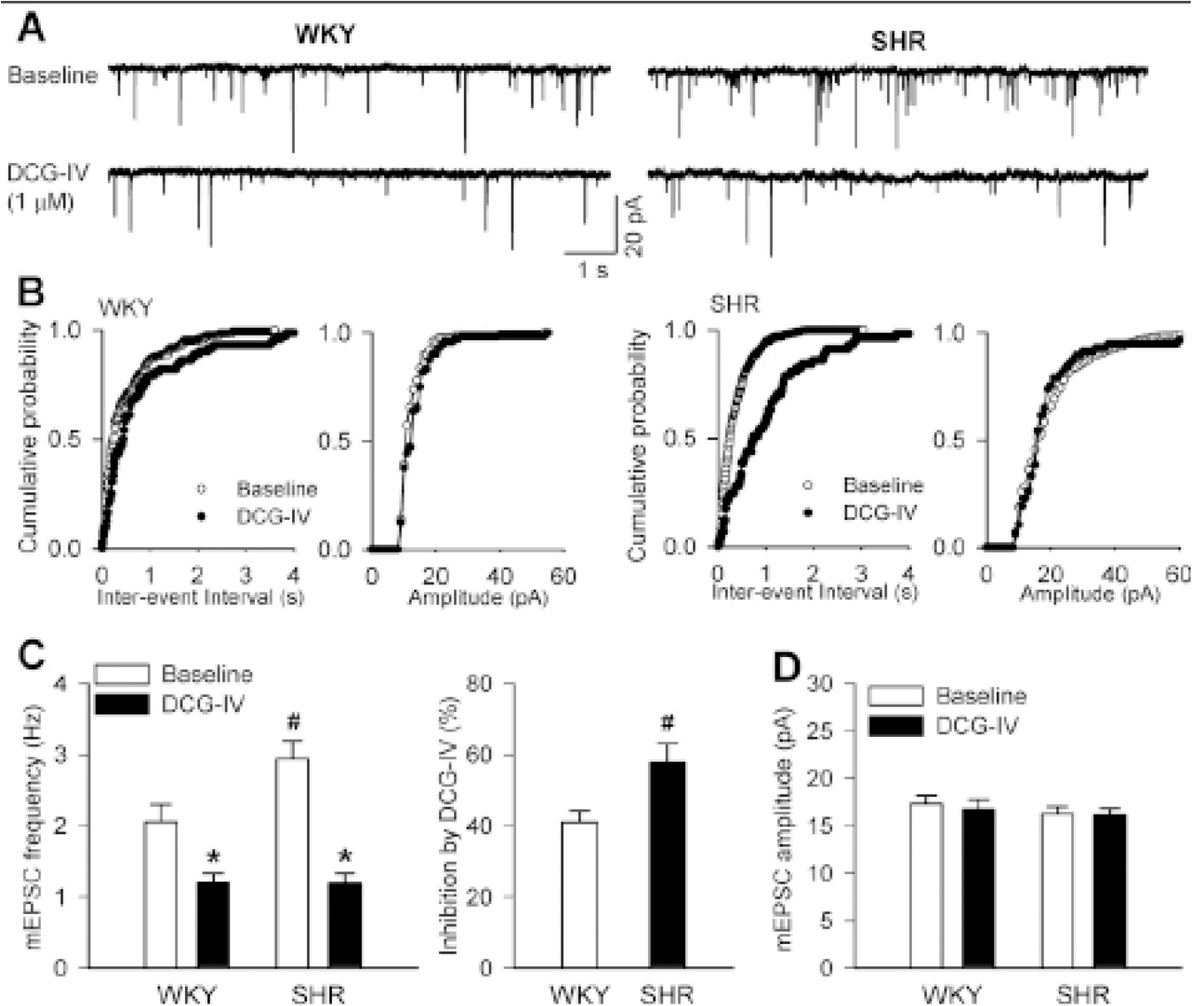


Figure 2. DCG-IV reduces the frequency of mEPSCs of spinally projecting PVN neurons more in SHRs than in WKY rats

A, Original recordings show mEPSCs recorded from labeled PVN neurons during control and application of DCG-IV (1 μ M) in 1 WKY rat and 1 SHR. B, Cumulative probability plots from the same neuron in A show the distribution of interevent interval and amplitude of mEPSCs during control and DCG-IV application. C and D, Summary data show the effect of DCG-IV (1 μ M) on the frequency and amplitude of mEPSCs of labeled PVN neurons in WKY rats and SHRs (n = 8 neurons in each group). * P < 0.05, compared with the respective baseline value. # P < 0.05, compared with the corresponding value in WKY rats.

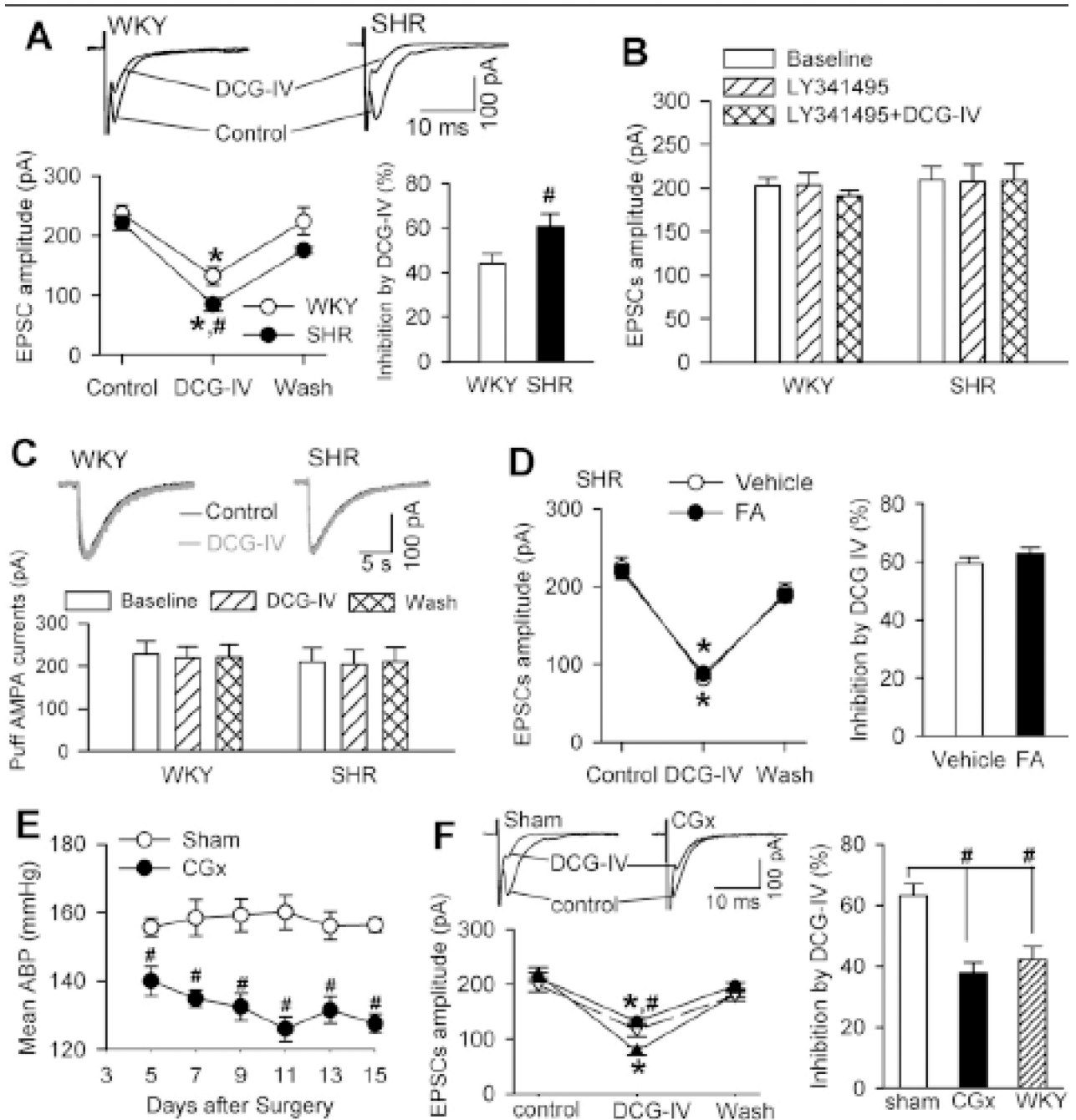


Figure 3. DCG-IV inhibits synaptically evoked EPSCs of spinally projecting PVN neurons more in SHRs than in WKY rats

A, Original traces and group data show the effects of DCG-IV (1 μ M) on electrically evoked EPSCs of labeled PVN neurons recorded with intracellular dialysis of 1 mM GDP- β -s in SHRs and WKY rats (n = 8 neurons in each group). B, Summary data show LY341495 blocked the effect of DCG-IV on the amplitude of evoked EPSCs in WKY rats (n = 7) and SHRs (n = 8). C, Original recordings and summary data show the effect of DCG-IV on AMPA receptor currents elicited by AMPA puff application in WKY rats and SHRs (n = 7 in each group). D, Summary data show the effect of DCG-IV on evoked EPSCs of labeled PVN neurons in vehicle- and fluoroacetate (FA)-treated brain slices obtained from SHRs (n

= 7 neurons in each group). E, Group data show the time course of changes in mean ABP of SHRs subjected to sham surgery or CGx (n = 5 rats in each group). F, Original traces and summary data comparing DCG-IV–induced inhibition of evoked EPSCs in SHRs undergoing CGx (n = 8 neurons) and sham controls (n = 7 neurons). The effect of DCG-IV on evoked EPSCs in WKY rats (n = 8 neurons) was also plotted as the control. * P < 0.05, compared with the respective baseline control. # P < 0.05, compared with the corresponding value in WKY rats or sham controls.

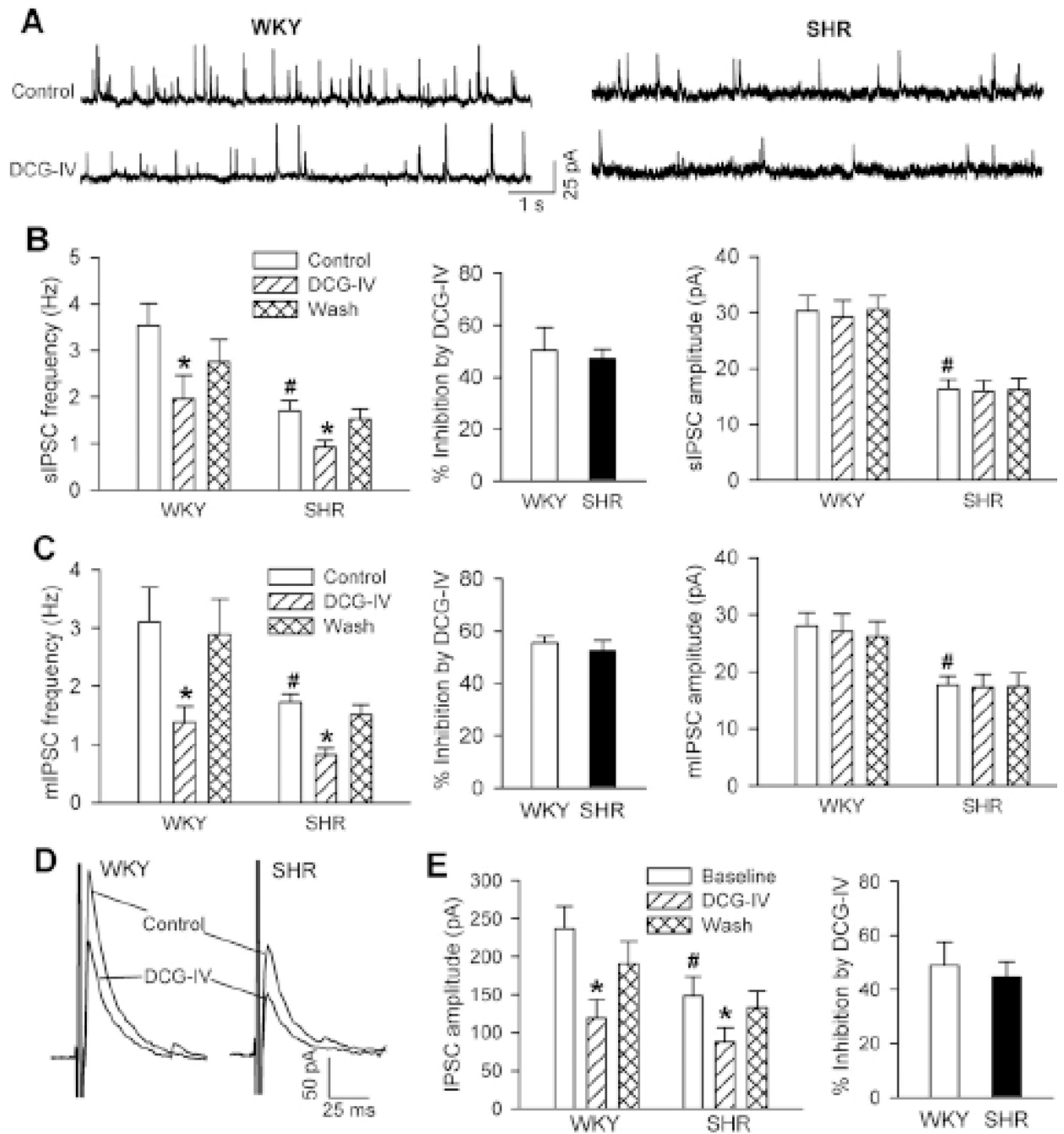


Figure 4. DCG-IV similarly reduces GABAergic IPSCs of spinally projecting PVN neurons in WKY rats and SHRs

A, Original traces show sIPSCs of labeled PVN neurons during control and application of 1.0 μ M DCG-IV in 1 WKY rat and 1 SHR. B, Summary data show the effect of DCG-IV on the frequency and amplitude of sIPSCs in WKY rats (n = 8) and SHRs (n = 11). C, Group data show the effect of DCG-IV on the frequency and amplitude of mIPSCs in WKY rats (n = 8) and SHRs (n = 7). D, Representative recordings of evoked IPSCs of labeled PVN neurons during control and the application of 1 μ M DCG-IV in 1 WKY rat and 1 SHR. E, Summary data show the effect of DCG-IV on evoked IPSCs in WKY rats and SHRs (n = 8)

in each group). * $P < 0.05$, compared with the respective baseline control. # $P < 0.05$, compared with the corresponding value in WKY rats.

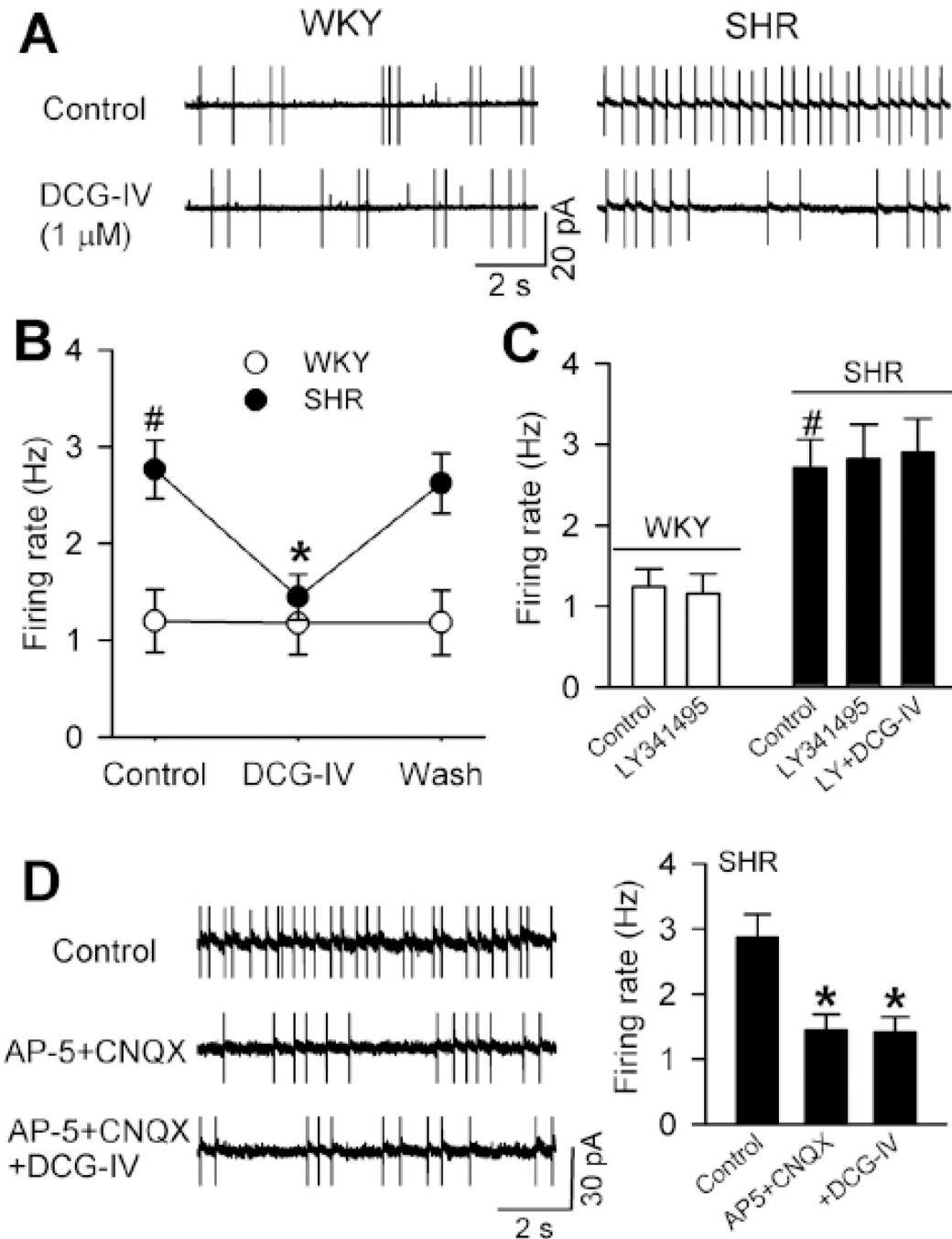


Figure 5. DCG-IV differentially inhibits the firing of spinally projecting PVN neurons in SHR

A, Original cell-attached recordings show the firing activity of labeled PVN neurons during control and application of 1 μ M DCG-IV in 1 WKY rat and 1 SHR. B, Summary data show DCG-IV reduced the firing rate of labeled PVN neurons in SHR but not in WKY rats ($n = 10$ in each group). C, Group data show LY341495 blocked the effect of DCG-IV on the firing activity of PVN neurons in SHR ($n = 8$). LY341495 had no effect on the basal firing of PVN neurons in WKY rats ($n = 6$). D, Original traces and summary data show that DCG-IV had no further effect on the firing of PVN neurons of SHR in the presence of AP-5 and CNQX ($n = 6$). * $P < 0.05$, compared with the baseline control. # $P < 0.05$, compared with the corresponding value in WKY rats.

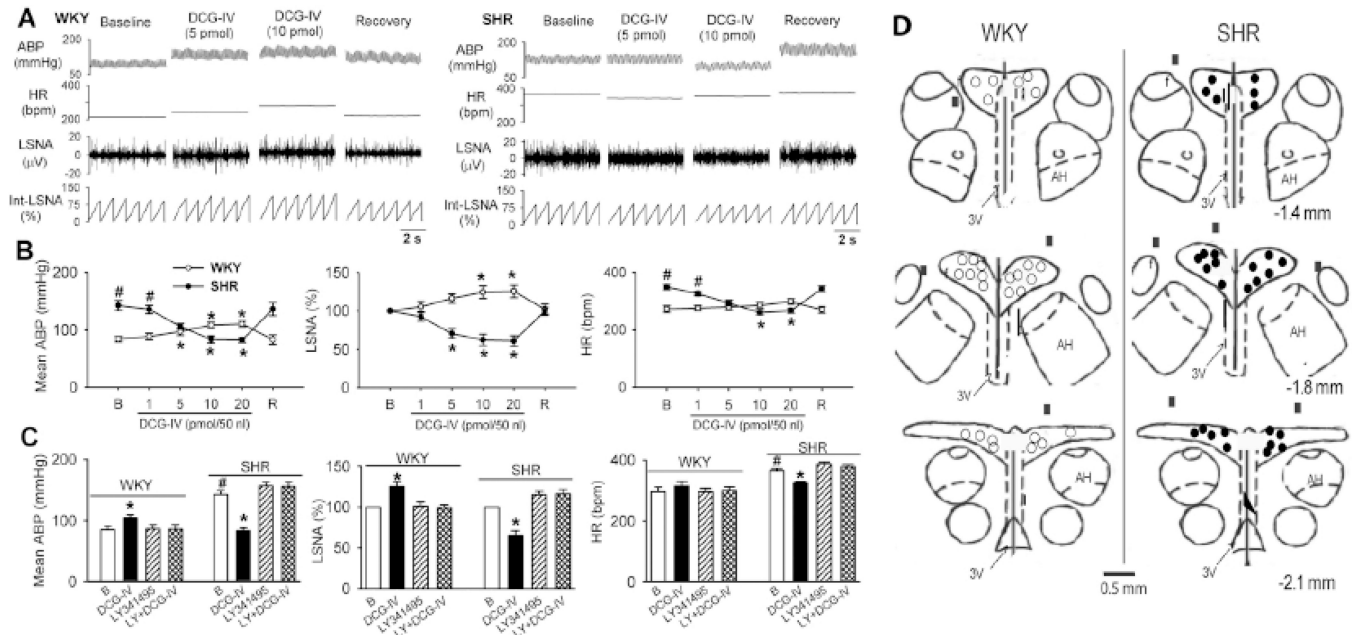


Figure 6. Microinjection of DCG-IV into the PVN inhibits sympathetic vasomotor tone in SHRs
 A, Representative recordings show the effect of bilateral microinjection of DCG-IV (5 and 10 pmol/50 nl) into the PVN on ABP, HR, LSNA, and integrated LSNA (Int-LSNA) in 1 SHR and 1 WKY rat. B, Summary data show differential effects of microinjection of DCG-IV (1–20 pmol/50 nl) into the PVN on LSNA, mean ABP, and HR in SHRs and WKY rats ($n = 7$ in each group). C, Group data show that microinjection of LY341495 (6 pmol/50 nl) blocked the effect of DCG-IV on LSNA, mean ABP, and HR in WKY rats and SHRs ($n = 7$ in each group). B, baseline; R, recovery; LY, LY341495. * $P < 0.05$ compared with the corresponding baseline control. # $P < 0.05$, compared with the corresponding value in WKY rats. D, Schematic drawings show the microinjection sites in WKY rats and SHRs. Note that misplacement of microinjections was marked with filled squares. The distance posterior to the bregma is shown below the diagram in each panel. AH, anterior hypothalamus; 3V, the third ventricle.