Involvement of enhanced cardiac sympathetic afferent reflex in sympathetic activation in early stage of diabetes

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Zhang L, Xiong XQ, Fan ZD, Gan XB, Gao XY, Zhu GQ. Involvement of enhanced cardiac sympathetic afferent reflex in sympathetic activation in early stage of diabetes. J Appl Physiol 113: 47–55, 2012. First published May 10, 2012; doi:10.1152/japplphysiol.01228.2011.—Cardiac sympathetic afferent reflex (CSAR) is involved in sympathetic activation. The present study was designed to investigate the contribution of enhanced CSAR to sympathetic activation in the early stage of diabetes and the involvement of AT1 receptors in the paraventricular nucleus (PVN). Diabetes was induced by a single intravenous injection of streptozotocin in rats. Acute experiments were carried out under anesthesia after 3 wk. The CSAR was evaluated by the responses of renal sympathetic nerve activity (RSNA) and mean arterial pressure (MAP) to epicardial application of capsaicin or bradykinin. Sympathetic activity and CSAR were enhanced in diabetic rats. Plasma norepinephrine and angiotensin II were increased, but the transient receptor potential vanilloid 1 (TRPV1) in the left ventricle wall was not significantly increased in diabetic rats. Pericardial injection of resiniferatoxin to desensitize cardiac afferents or PVN microinjection of lidocaine attenuated the CSAR and decreased the RSNA and MAP in diabetic rats. The AT1 receptor expression in the PVN increased in diabetic rats. Angiotensin II in the PVN caused greater increases in the RSNA and MAP and enhancement in the CSAR in diabetic rats, which were abolished by the losartan pretreatment. Losartan decreased the RSNA and MAP and attenuated the CSAR in diabetic rats but not in control rats. These results indicate that the CSAR is enhanced in the early stage of diabetic rats, which contributes to the sympathetic activation. AT1 receptors in the PVN are involved in the enhanced CSAR in diabetic rats.

diabetes; sympathetic activity; paraventricular nucleus; cardiovascular reflex; angiotensin

AUTONOMIC NEUROPATHY is one of the most common complications of diabetes and is characterized by sympathetic dominance and parasympathetic withdrawal (12, 23). The imbalance of the autonomic nervous system leads to a wide range of disorders such as silent myocardial infarction, cardiac arrhythmias, ulceration, gangrene, and nephropathy. Cardiac autonomic neuropathy increases morbidity and mortality in diabetes and may have greater predictive power than traditional risk factors for cardiovascular events (33). Autonomic neuropathy is also associated with an increased risk of sudden death (32). Exercise training improves aerobic capability of insulin-deficient rats without changing cardiovascular characteristics associated with the parasympathetic nervous system (37). The enhanced sympathetic drive promotes a higher risk of arrhythmias, a procoagulant state, a trophic effect with left ventricular and arteriolar hypertrophy, and an atherosclerotic damage due to increased pulsatile stress, insulin resistance, and vasoconstriction (21). The symptoms and signs of diabetic autonomic neuropathy carry a poor prognosis in diabetic patients (12).

It is known that the activation of the cardiac sympathetic afferent reflex (CSAR) increases sympathetic outflow and arterial pressure in dogs (15), rats (43), and cats (10). The CSAR is induced by epicardial application of bradykinin, capsaicin, hydrogen peroxide, or adenosine, or by endogenous chemicals such as bradykinin and adenosine released from myocardium during myocardial ischemia (6, 16). It is enhanced in chronic heart failure due to the increased central gain of the reflex and the cardiac sympathetic afferent activities (14, 35). The enhanced CSAR contributes to the sympathetic overdrive in chronic heart failure (36). However, it is unknown whether the CSAR is involved in the sympathetic overdrive in the early stage of diabetes.

Paraventricular nucleus (PVN) is known to play an important role in regulating the sympathetic outflow and cardiovascular activity via its projections to the intermediolateral column of the spinal cord and rostral ventrolateral medulla (1). Our previous studies have shown that the PVN is a key component of the central neurocircuitry of the CSAR (41). Angiotensin II (ANG II) and AT1 receptors in the PVN contribute to the enhanced CSAR and sympathetic activation in rats with chronic heart failure (42) and renovascular hypertension (3). The present study is designed to determine whether the CSAR contributes to the sympathetic overdrive and whether the AT1 receptors in the PVN are involved in the enhanced CSAR in the early stage of diabetic rats.

METHODS

Induction of Diabetes

Male Sprague-Dawley rats 8–9 wk old weighing between 280 and 300 g were used to induce diabetes with streptozotocin (STZ), which causes destruction of the beta cell in pancreatic islets and mimics the progression of type 1 diabetes clinically (28). STZ was dissolved in 0.1 M of sodium citrate buffer adjusted to a pH of 4.5. After being fasted for 8 h, the rats were randomly subjected to a single injection of vehicle or STZ (60 mg/kg) via the tail vein (25). Diabetes was identified by evaluating with a glucometer (Lifescan, Milpitas, CA) in accordance with the inclusion criteria (blood glucose level > 300 mg/dl). Onset of diabetes was characterized by hyperglycemia, polyphagia, and polyuria. All the rats underwent blood glucose evaluation to ensure the maintenance of diabetes prior to the acute experiments. Four STZ-treated rats were excluded because of unqualified blood glucose level. All vehicle-treated rats showed a normal blood glucose level. The experimental procedures were approved by the Experimental Animal Care and Use Committee of Nanjing Medical University and complied with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23, revised 1996).
rats were caged in a controlled temperature and humidity with a 12:12-h light/dark cycle. Standard laboratory chow and drinking water were available ad libitum.

**General Procedures of Acute Experiment**

Acute experiments were carried out at the end of the 3rd week after the injection of STZ or vehicle. The rats were anesthetized with urethane (800 mg/kg) and α-chloralose (40 mg/kg) intraperitoneally. Adequate depth of anesthesia was evaluated by the absence of corneal reflexes and paw withdrawal response to a noxious pinch. Supplemental doses of anesthetic agents were used to maintain appropriate depth of anesthesia during the experiment. A midline incision was made to expose the trachea and carotid artery. The trachea was connected to a rodent ventilator (683; Harvard Apparatus) for mechanical ventilation. A catheter connected with a pressure transducer (MLT0380, ADInstruments) was placed into the right common carotid artery for the blood pressure recordings.

**Evaluation of Basal Sympathetic Activity**

Blood samples were collected from the carotid artery into a tube containing EDTA-K2, and the plasma norepinephrine level was determined by high-performance liquid chromatography (HPLC) with YWG-C18 column and electrochemical detection (Waters 2465, Milford, MA) (26). Then the maximal depressor response to intravenous injection of hexamethonium hydrochloride (30 mg/kg), a ganglionic blockade, was used as an index of sympathetic activity (18).

**RSNA Recordings**

The left renal sympathetic nerve was exposed through a retroperitoneal incision and cut distally to abolish its afferent activity. The nerve was placed on a pair of silver electrodes and immersed in mineral oil. The renal sympathetic nerve activity (RSNA) was amplified with an AC/DC differential amplifier (3000, A-M System WA) and filtered with a band-pass between 60 and 3,000 Hz. The RSNA was integrated with a time constant 100 ms. The raw RSNA, integrated RSNA, arterial blood pressure (ABP), mean arterial pressure (MAP), and heart rate (HR) were simultaneously recorded with a PowerLab data-acquisition system (8SP, ADInstruments). The background noise of the recorded signals was determined after section of the central end of the nerve at the end of the experiment and subtracted from the integrated RSNA values.

**Evaluation of CSAR**

A left lateral thoracotomy was performed to expose the heart, and the pericardium was removed. The CSAR was elicited by epicardial application of a piece of filter paper (3 mm × 3 mm) containing capsaicin (0.1 nmol or 1.0 nmol in 2.0 μl) or bradykinin (0.03 nmol or 0.3 nmol in 2.0 μl) to the anterior wall of the left ventricle for 1 min. After the filter paper was removed, the epicardium was rinsed three times with 10 ml of normal saline (37°C). Successive applications of capsaicin or bradykinin were separated by at least 30 min to avoid tachyphylaxis. The CSAR was evaluated by the RSNA and MAP response to the epicardial application of capsaicin or bradykinin (3, 11).

**PVN Microinjection**

The rat was fixed in a stereotaxic frame (Stoelting, Chicago, IL). The stereotaxic coordinates of the PVN are 1.8 mm caudal from bregma, 0.4 mm lateral to the midline, and 7.9 mm ventral to the dorsal surface according to Paxinos and Watson’s rat atlas (20a). The microinjection volume was 50 nl for each side of the PVN. The bilateral microinjections were simultaneously carried out and completed within 1 min. At the end of the experiment, the same volume of Evans blue dye was injected into the microinjection site for histological identification. Six rats were excluded from data analysis because the microinjection sites were near the boundary of the PVN or outside the PVN (Fig. 1).

**Measurement of Plasma ANG II**

Plasma ANG II was measured with commercial ELISA kits (R&D systems, Minneapolis, MN) according to the manufacturer’s descriptions. The 96-well plates were incubated with antibodies specific for rat ANG II. Samples and standard diluent buffer were added, incubated, and washed. Horseradish peroxidase-conjugated solution was added and then washed out. The reactions were stopped with stop solution, and the final solution was read at 450 nm using a microplate reader (ELX800, BioTek Instruments) (7).

**Measurement of Transient Receptor Potential Vanilloid 1 in the Left Ventricle Wall**

The left ventricle was cut into pieces, homogenized in grinders embedded in ice, and centrifuged 20 min at 2,000 rpm. Transient receptor potential vanilloid 1 (TRPV1) expression in the supernatant was evaluated by commercial ELISA kits following the manufacturer’s instructions (R&D Systems). Briefly, after standard wells were set, samples and dilution were added, incubated for 30 min at 37°C and then washed. HRP-conjugated solution was added and then washed. The color reaction was developed and the absorbance was read at 450 nm.

**Measurement of AT1 Receptor Expression in PVN**

AT1 receptor protein in the PVN was measured with Western blotting as previously reported (26). The total protein was measured using a protein assay kit (BCA, Pierce). The protein samples were loaded on a 10% SDS-PAGE for electrophoresis and then blotted to a nitrocellulose membrane (Pall, Pensacola, FL) and incubated overnight with primary polyclonal anti-rabbit antibody to AT1 receptors (1:300, Santa Cruz Biotechnology, Santa Cruz, CA). After incubation with goat anti-rabbit IgG (1:5,000, Santa Cruz Biotechnology), the bands were visualized with enhanced chemiluminescence (Pierce) and exposed to BioMax films (Kodak). Then the membranes were stripped and incubated with primary antibody to β-actin (1:300, Santa Cruz Biotechnology) and rabbit anti-mouse secondary antibody (1:5,000, Santa Cruz Biotechnology). The results were expressed as the ratio of the optical density of the interesting band relative to β-actin band.
Protocols

Protocol 1. Blood samples were collected for measuring plasma norepinephrine and ANG II. The brain and heart were removed for determining the AT1 receptor expression in the PVN and the TRPV1 expression in the left ventricle wall in control and diabetic rats, respectively (n = 6 for each group).

Protocol 2. The maximal depressor effect of intravenous injection of ganglionic blockade, hexamethonium hydrochloride, was determined in control and diabetic rats as an index of basal sympathetic tone (n = 6 for each group).

Protocol 3. The CSAR was evaluated by the RSNA and MAP responses to randomly epicardial applications of saline, capsaicin (0.1 and 1.0 nmol), or bradykinin (0.03 and 0.3 nmol) in control and diabetic rats (n = 8 for each group). The interval between epicardial applications was at least 30 min.

Protocol 4. Resiniferatoxin (RTX) was used to determine the effects of cardiac afferent block on the RSNA and MAP. Either control or diabetic rats were randomly divided into two groups, which were respectively subjected to injection of RTX (60 pmol) or saline into the pericardial cavity (n = 6 for each group). At the 140th minute after administration of RTX or saline, the CSAR induced by capsaicin (1.0 nmol) was determined to confirm the effectiveness of RTX on blocking the cardiac afferents.

Protocol 5. To determine the role of the PVN in the enhanced CSAR and sympathetic activation, the PVN microinjection of saline, lidocaine (8.5 nmol), and saline were in turn carried out in control and diabetic rats (n = 6 for each group). The interval between microinjections was at least 30 min for complete recovery. The CSAR induced by capsaicin (1.0 nmol) was determined at the 5th minute after PVN microinjection.

Protocol 6. To determine the roles of ANG II and AT1 receptors in the PVN, either control or diabetic rats were randomly divided into four subgroups, which were subjected to the PVN microinjection of saline, ANG II (0.3 nmol), losartan (50 nmol), and same dose of ANG II pretreated with losartan (n = 6 for each group). The CSAR induced by capsaicin (1.0 nmol) was determined at the 5th minute after PVN microinjection.

Drugs

ANG II, capsaicin, bradykinin, STZ, losartan, and lidocaine were obtained from Sigma Chemical.

Statistical Analysis

Student’s t-test was used for comparisons between the control and diabetic rats. Student’s paired t-test was used for comparisons between two observations in the same animal. One-way ANOVA followed by the Bonferroni test for post hoc analysis was used when multiple comparisons were made. All data were expressed as means ± SE. P < 0.05 was considered statistically significant.

RESULTS

General Data

The blood glucose in diabetic rats was significantly higher than that in control rats, while body weight in diabetic rats was significantly lower than that in control rats (Table 1). There was no significant difference between MAP and HR in control and diabetic rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>367.0 ± 8.3</td>
<td>276.9 ± 6.5*</td>
</tr>
<tr>
<td>Blood glucose, mg/dl</td>
<td>112.3 ± 3.8</td>
<td>467.1 ± 32.7*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>92.8 ± 2.8</td>
<td>87.4 ± 2.2</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>369.4 ± 9.1</td>
<td>345.9 ± 11.3</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. MAP, mean arterial pressure; HR, heart rate. *P < 0.05 compared with control rats.

Basal Sympathetic Activity and Plasma ANG II

Both plasma norepinephrine level and the maximal depressor response to intravenous administration of the angiglionic blockade were used as indexes of basal sympathetic activity at the end of the 3rd week. The plasma norepinephrine and ANG II levels were higher in diabetic rats than that in control rats (Fig. 2A). There was no significant difference in the baseline MAP between the diabetic rats and control rats, but the maximal depressor response to hexamethonium hydrochloride was greater in diabetic rats than that in control rats (Fig. 2B).

CSAR Induced by Capsaicin and Bradykinin

Representative recordings show the RSNA and MAP responses to epicardial application of capsaicin in control and diabetic rats (Fig. 3A). The CSAR induced by capsaicin was significantly enhanced in diabetic rats compared with that in control rats. High dose of capsaicin caused greater RSNA and MAP responses than low dose of capsaicin. Bradykinin-induced CSAR was similar to capsaicin-induced CSAR in both control and diabetic rats (Fig. 3B).

TRPV1 in Left Ventricle Wall

There was no significant difference of TRPV1 in the left ventricle wall between diabetic rats and control rats (14.0 ± 1.6 vs. 10.8 ± 0.9 ng/mg protein, P = 0.14).
Intrapericardial administration of RTX caused immediate and short-term increases in both RSNA and MAP, peaking at 10 min in both control and diabetic rats. The RTX-caused RSNA and MAP changes in diabetic rats were greater than those in control rats. Subsequent decreases in RSNA and MAP were only found in diabetic rats but not in control rats. The RTX treatment abolished the CSAR evoked by capsaicin in both control and diabetic rats. Intrapericardial administration of saline had no significant effects on RSNA, MAP, and CSAR (Fig. 4).

Effects of RTX

Fig. 3. Cardiac sympathetic afferent reflex (CSAR) in control rats and diabetic rats. A: representative recordings showing the capsaicin-induced CSAR was enhanced in diabetic rats at the end of the 3rd week. ABP, arterial blood pressure; RSNA, renal sympathetic nerve activity. B: the CSAR induced by epicardial application of saline and 2 doses of capsaicin or bradykinin in control and diabetic rats at the end of the 3rd week. Values are means ± SE; n = 8 for each group. *P < 0.05 vs. saline; †P < 0.05 vs. low dose of capsaicin or bradykinin; ‡P < 0.05 vs. control rats.
Effects of Lidocaine in the PVN

The PVN microinjection of lidocaine decreased the baseline RSNA and MAP in diabetic rats but not in control rats. However, lidocaine in the PVN abolished the CSAR in both diabetic and control rats (Fig. 5).

Effects of ANG II and Losartan in the PVN

Microinjections of ANG II into the PVN caused more increases in the baseline RSNA and MAP and greater enhancement in the CSAR in diabetic rats than that in control rats, which was abolished by the pretreatment with losartan in the PVN. Microinjections of losartan into the PVN decreased the RSNA and MAP and normalized the enhanced CSAR in diabetic rats but had no significant effects in control rats (Fig. 6).

AT1 Receptor Expression in the PVN

The AT1 receptor expression in the PVN was increased in diabetic rats compared with control rats (Fig. 7).

DISCUSSION

The primary new findings in the present study are that the CSAR is enhanced in the early stage of streptozotocin-induced diabetic rats, which contributes to the sympathetic activation, and that the AT1 receptors in the PVN are involved in the enhanced CSAR and sympathetic outflow.

Plasma norepinephrine is inferred as a consequence of enhanced neurotransmitter release secondary to the increased sympathetic neuronal activity (9, 13). The hexamethonium-induced maximal decrease in the MAP is an index to evaluate the baseline peripheral sympathetic activity (18). In the present study, higher plasma norepinephrine and greater depressor response to hexamethonium indicated excessive sympathetic activity at the end of the 3rd week in this model of diabetes. It is particularly worth noting that the CSAR caused by capsaicin was enhanced in diabetic rats. The enhanced CSAR may be involved in the excessive sympathetic activation, which is deleterious in diabetes over the long term. It has been reported that cardiac bradykinin B1 and B2 receptor mRNA expression is increased in rats with STZ-induced diabetes (27). Transgenic activation of the kallikrein-kinin system inhibits intramyocardial inflammation, oxidative stress, and endothelial dysfunction in experimental diabetic cardiomyopathy, implying a protective role for bradykinin signaling (30). However, complete loss of bradykinin expression does not worsen cardiac function or increase myocardial fibrosis in diabetes. The possible explanation is that the B1 receptors are proinflammatory and the B2 receptors promote cardioprotection and thus the impact of loss of both receptors may be relatively neutral (39).
been reported that cardiac bradykinin elicits a sympathoexcitatory reflex by epicardial B2 receptors in rats (31). In the present study, the RSNA and MAP response to epicardial application of bradykinin was greater in diabetic rats than control rats, confirming that the CSAR was enhanced in diabetic rats.

RTX is an ultrapotent analog of capsaicin with the property of binding TRPV1, which is a cation channel with ligand-gated, nonselective characters and is preferentially distributed in primary afferent neurons with small diameter, such as nociceptive sensory nerves. The RTX is used as a convenient way to deplete capsaicin-sensitive afferent fibers (22, 40). It has been found that the cardiac afferents containing TRPV1 are essential for the CSAR (40). In the present study, intrapericardial administration of RTX caused immediate and short-term increases in the RSNA and MAP in both diabetic and control rats, but the RSNA and MAP responses to RTX were greater in diabetic rats than control rats. The excitatory effects of RTX can be explained as activating the TRPV1-containing cardiac afferents to evoke the CSAR. The results suggested that the RTX-induced CSAR was enhanced in diabetic rats, which was similar to the capsaicin-induced CSAR that was enhanced in diabetic rats. It is particularly worth noting that RTX caused long-lasting decreases in the RSNA and MAP in diabetic rats but not in control rats. The inhibitory effects of RTX are attributed to the blockade of the TRPV1-containing cardiac afferents, which is supported by the finding that the CSAR cannot be induced by capsaicin examined at the 140th minute after RTX. The results indicate that the enhanced CSAR contributed to the excessive sympathetic activity in diabetic rats.

The PVN is an important component of the central pathway of the CSAR in rats (41). Microinjections of lidocaine in discrete brain areas can serve as a tool for reversible functional inactivation (8, 24). In the present study, microinjection of lidocaine into the PVN abolished the CSAR in both diabetic and control rats, and decreased the RSNA and MAP in diabetic rats but not in control rats. The results suggest the importance of the PVN in regulating the CSAR and sympathetic activity in diabetic rats, and reinforce the conclusion drawn from the RTX that enhanced CSAR contributes to the sympathetic activation in diabetic rats.

Emerging studies have shown that ANG II increases the activity of neurons in the PVN. Chronic subcutaneous infusion of ANG II causes marked and sustained activation in the PVN (5). The blockade of AT1 receptors in the PVN reduces central hyperosmolality-induced sympathetic activation (4). The blood-borne ANG II upregulates brain AT1 receptor in the PVN and subfornical organ (SFO) by activating intracellular p44/42 MAPK and JNK signaling pathways (38). Our previous studies have shown that ANG II in the PVN regulates the sympathetic outflow and CSAR, which is mediated by AT1 receptors in normal rats (43). The AT1 receptors in the PVN are involved in the enhanced CSAR and sympathetic activation in

![Fig. 5. Effects of PVN microinjection of saline or lidocaine on the baseline RSNA and MAP and the CSAR in control and diabetic rats at the end of the 3rd week. Values are means ± SE; n = 6 for each group. *P < 0.05 vs. saline or the values at the 30th min after lidocaine; †P < 0.05 vs. control rats.](http://jap.physiology.org/content/111/3/1228/F5)
rats with chronic heart failure (34) and hypertension (3). Artificial microRNA interference targeting the AT1a receptors in the PVN attenuates the CSAR, sympathetic activity, and hypertension (7). Superoxide anions in the PVN mediate the enhanced CSAR and sympathetic activity in hypertensive rats (11). In this study, ANG II in the PVN caused greater increases in the RSNA and MAP and enhancement in the CSAR in diabetic rats, which were attenuated by the pretreatment with losartan. The PVN microinjection of losartan decreased the RSNA and MAP and inhibited the enhanced CSAR in diabetic rats but not in control rats. Furthermore, the expression of AT1 receptors in the PVN was increased in diabetic rats. The results indicate that ANG II and AT1 receptors in the PVN are involved in the enhanced CSAR and sympathetic overdrive in diabetic rats.

One mechanism leading to the enhanced CSAR is the increased central gain of the reflex in diabetic rats, which is supported by the findings that abolishing the CSAR with lidocaine in the PVN decreases the RSNA and MAP in diabetic rats but not in control rats. Furthermore, the expression of AT1 receptors in the PVN was increased in diabetic rats. The results indicate that ANG II and AT1 receptors in the PVN are involved in the enhanced CSAR and sympathetic overdrive in diabetic rats.

One mechanism leading to the enhanced CSAR is the increased central gain of the reflex in diabetic rats, which is supported by the findings that abolishing the CSAR with lidocaine in the PVN decreases the RSNA and MAP in diabetic rats, and that the AT1 receptors in the PVN may be responsible for the increased central gain of the CSAR. On the other hand, blockade of the TRPV1-containing cardiac afferents with RTX caused long-lasting decreases in RSNA and MAP in diabetic rats but not in control rats, suggesting a possibility that the sensitization of the cardiac sympathetic afferents may be one mechanism leading to the CSAR enhancement. It is interesting that there was no significant difference of TRPV1 in the left ventricle wall between diabetic rats and control rats, indicating that the enhanced CSAR in diabetic rats do not result from the changes of TRPV1 expression. Myocardial ischemia due to decreased cardiac output (2), cardiomyopathy (17), and hypovolemia (19) may increase the production of endogenous

Fig. 6. Effects of the PVN microinjection of saline, ANG II, losartan, and ANG II pretreated with losartan on the baseline RSNA and MAP and the CSAR at the end of the 3rd week. Values are means ± SE; n = 6 for each group. *P < 0.05 vs. saline; †P < 0.05 vs. control rats; ‡P < 0.05 vs. ANG II alone.

Fig. 7. AT1 receptor expression in the PVN in control and diabetic rats at the end of the 3rd week. The AT1 receptor protein was determined with Western blot and was expressed as the ratio to β-actin. Values are means ± SE; n = 6 for each group. *P < 0.05 vs. control.
chemicals such as bradykinin, prostaglandins, superoxide anions, and hydrogen peroxide. These chemicals stimulate cardiac sympathetic afferents and thus enhance the CSAR.

It is interesting that the sympathetic activity and plasma norepinephrine were increased, but no change was found in the HR and MAP during early diabetes, which is consistent with other studies on STZ-induced diabetic rats under both conscious and anesthetized conditions (2, 29). The reduced movements may be one reason for unchanged HR. The decreased cardiac output due to a myocardial dysfunction (2) and the hypovolemia secondary to hyperglycemia and resultant osmotic diuresis (19) may contribute to the lack of conformity between the sympathetic activity and blood pressure. The autonomic function changes often occur before or without alterations in blood pressure and HR, and it is unwise to treat blood pressure and HR as indexes for evaluating the tonic sympathetic outflow, for they are uncorrelated with autonomic neural function (20). The enhanced CSAR and sympathetic activity may be helpful in the maintenance of blood pressure, ventricular function, and cardiac output during the early stage of diabetes, which is supported by the present findings that inhibition of the CSAR and sympathetic activity results in a greater decrease in MAP in diabetic rats than normal rats. Without the sympathetic activation, a hypotensive state would have occurred because of the decreased cardiac output due to myocardial dysfunction, hypovolemia due to osmotic diuresis, and impairment of innervation of heart and blood vessels in diabetes (2, 19). Despite its beneficial effects in the earlier stage of diabetes, long-term excessive sympathetic activity stimulates renin-angiotensin activity and promotes sodium reabsorption, thus increasing cardiovascular risk and complications (21).

In conclusion, the CSAR is enhanced in the early stage of STZ-induced diabetic rats, which contributes to the sympathetic activation. Blocking the cardiac afferents or inhibiting the activity of the PVN neurons abolished the CSAR and decreased the sympathetic outflow. The AT1 receptors in the PVN are involved in the enhanced CSAR in diabetic rats.

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES


