Original Article

Candesartan and Insulin Reduce Renal Sympathetic Nerve Activity in Hypertensive Type 1 Diabetic Rats

Chie TAKIMOTO[1)], Hiroo KUMAGAI[2)], Motohisa OSAKA[3)], Katsufumi SAKATA[1)], Toshiko ONAMI[1)], Tadashi KAMAYACHI[1)], Kamon IIGAYA[1)], Koichi HAYASHI[1)], Takao SARUTA[1)], and Hiroshi ITOH[1)]

The nonlinearity of cardiovascular regulation is higher in normal physiology, whereas several diseases are characterized by a reduction in this nonlinearity. Reduced nonlinearity of heart rate regulation is a robust risk factor for high mortality in patients with myocardial infarction. We investigated the changes in linear and nonlinear correlations of cardiovascular regulation after administering drugs in hypertensive diabetic rats. Type 1 diabetes was induced in rats by intraperitoneally injecting spontaneously hypertensive rats with streptozotocin. The animals were then divided into 4 groups and each group was given vehicle, candesartan, amlodipine, or insulin for 2 weeks. Blood pressure, heart rate, renal sympathetic nerve activity, and renal blood flow were simultaneously recorded in the conscious state, and the linear and nonlinear correlations were compared by using coherence and the mutual information method. Candesartan and amlodipine decreased blood pressure to a similar extent, but renal sympathetic nerve activity was significantly lower in the candesartan group than in the vehicle group. The renal sympathetic nerve activity in the insulin group was also lower than in the vehicle group. There were no significant differences in linear correlation among the 4 groups. In contrast, the nonlinear correlations between renal sympathetic nerve activity and blood pressure in the candesartan group and the insulin group were significantly higher than in the vehicle group. Candesartan and insulin decreased renal sympathetic nerve activity and increased the nonlinearity. These results suggest that reducing the activity of renin-angiotensin system and insulin that lowers blood glucose level may improve autonomic nervous system dysfunction and neurohumoral regulation of the cardiovascular system in diabetic hypertensive rats. (*Hypertens Res* 2008; 31: 1941–1951)

***Key Words*: hypertension, diabetes mellitus, nonlinear correlation, sympathetic nervous system, angiotensin II receptor blocker**

Introduction

While hypertension is an independent risk factor for cardiovascular disease and mortality (*1*), diabetes-induced dysfunction of the autonomic nervous system is also known to be an independent risk factor. Although this dysfunction is due to increased sympathetic nerve activity and reduced parasympathetic nerve activity—a state that is characterized by reduced heart rate (HR) variability (*2*)—linear and nonlinear correlations among blood pressure (BP), HR, renal sympathetic nerve activity (RSNA), and renal blood flow (RBF) have not been directly determined in diabetic humans or animal models. We therefore designed this study to examine how pronounced hyperglycemia against a background of hypertension affects sympathetic nervous control of the circu-

From the [1)]Divison of Endocrinology, Metabolism and Nephrology, Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan; [2)]Department of Nephrology, National Defense Medical College, Saitama, Japan; and [3)]Department of Basic Science, Faculty of Veterinary Science, Nippon Veterinary and Life Science University, Tokyo, Japan.

Address for Reprints: Chie Takimoto, M.D., Divison of Endocrinology, Metabolism and Nephrology, Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160–8582, Japan. E-mail: CZT17240@nifty.com

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lation and to identify drugs that are capable of improving the nonlinear correlations.

In a previous study (*3*) we used the "mutual information method" to quantify linear and nonlinear correlations between cardiovascular parameters (*4*, *5*). This method allowed us to demonstrate that the linear correlations between RSNA and BP and between RSNA and RBF were higher, and that the nonlinear correlations between them were lower, in spontaneously hypertensive rats (SHR) than in normotensive Wistar-Kyoto rats (WKY) (*3*).

In the present study we induced type 1 diabetes by injecting SHR with streptozotocin (STZ), which induces severe hyperglycemia, hypoinsulinemia, and glucose intolerance by destroying islet β cells (*6*, *7*). Although about 95% of diabetic patients have type 2 diabetes, hypertensive type 2 diabetic patients often exhibit metabolic syndromes and manifestations other than hyperglycemia, such as insulin-resistance, obesity, and hyperlipidemia. Since we consider hyperglycemia to be the most important diabetic factor affecting the sympathetic nervous system (SNS), we chose this simple type 1 diabetes model to assess the true effect of hyperglycemia on SNS.

Methods

BP, HR, RSNA, and RBF were simultaneously recorded in conscious rats, and we used a combination of coherence of transfer function (linear correlation) and mutual information (linear and nonlinear correlation) to determine whether the linear and nonlinear correlations between RSNA and BP and correlations between RSNA and RBF would change in type 1 diabetic rats given the angiotensin II receptor blocker (ARB), candesartan, or the calcium channel blocker (CCB) amlodipine orally for 2 weeks. Although several different types of antihypertensive therapies are effective against hypertension in diabetes (*8*), it remains unknown which drugs are effective in reducing the activity of SNS and increasing nonlinear correlations.

In addition, to examine the effects of diabetes and chronic insulin administration on the SNS, we investigated changes in the linear and nonlinear correlations between RSNA and BP and between RSNA and RBF after continuous insulin administration *via* subcutaneous mini-osmotic pumps for 2 weeks. Acute insulin injection appears to activate the SNS (*9*), and we tried to investigate the impact of lowering blood glucose *via* chronic insulin administration on sympathetic nerve activity.

Recording of BP, ECG, HR, RSNA, and RBF

Male 7-week-old SHRs were purchased from Sankyo Lab Service, Tokyo. All experimental procedures were performed in accordance with institutional animal care guidelines. Rats were allowed to rest for 1 week in a quiet room maintained at a constant temperature under a 12-h light/dark cycle, and they were given free access to normal-sodium rat chow (0.38% NaCl, Nippon Clea, Tokyo, Japan) and tap water. At 8 weeks of age, rats were intraperitoneally injected with a 60 mg/kg dose of STZ to create diabetic hypertensive rats, *i.e.*, SHR-STZ. A small amount of blood was drawn from the tail vein 2 weeks later to measure the glucose level and confirm hyperglycemia. Candesartan (1 mg/kg/d orally), amlodipine (5 mg/kg/d orally), rapid insulin (8 units/ kg/d *via* ALZET subcutaneous mini-osmotic pumps), or vehicle was given for 2 weeks starting at 13 weeks of age.

The rats in all experiments were used at 15 weeks of age. The nonfasting blood glucose level, insulin level, and body weight of each rat were measured at the beginning of the experiment. Before surgery, rats were deeply anesthetized with sodium pentobarbital, 30 mg/kg i.v., and supplemental doses of 10 mg/kg were injected as needed. A polyethylene catheter (PE-50; Clay Adams [Becton Dickinson], Franklin Lakes, USA) was placed in the femoral vein for drug administration. BP was measured directly through a catheter placed in the femoral artery and connected to a pressure transducer (TP-200T; Nihon Kohden Co., Tokyo, Japan) and polygraph (AP-611G; Nihon Kohden Co.).

We used a telemetry system to record ECG and monitor the HR of conscious, unrestrained rats. The transmitter (TA10EA-F20; Data Science, St. Paul, USA) was implanted into the peritoneal cavity 1–2 d before the experiment, and the receiver (RPC1; Data Science) was placed beneath the cage. The raw analog ECG data were digitized with an A/D converter (Power Lab; ADI, Rose Park, Australia) and converted to HR data.

Multifiber recordings of RSNA were performed as described previously (*3*, *10*, *11*). The left renal artery and vein were exposed under a microscope by retroperitoneal approach. The renal nerves were carefully isolated, and polytetrafluoroethylene-coated stainless wire electrodes (A-M System, Inc., Carlsborg, USA) were placed on the left renal nerve fascicle. A pulsed Doppler flow probe (inner diameter=1.0 mm, 20 MHz; CBI Co., Northborough, USA) was placed on the left renal artery to measure the RBF. The nerve fascicle, electrodes, left renal artery, and Doppler probe were covered with SilGel 604A and 604B (Wacker-Chemie, Munich, Germany).

Data Acquisition and Data Analysis

A minimum of 24 h later, the conscious diabetic rat was placed in a cage. The neural recording electrodes were connected to a high-impedance probe (JB101J; Nihon Kohden Co.) and then to a differential amplifier with a band-pass filter of 50 to 1,000 Hz, and visualized on an oscilloscope. The neurogram was rectified and integrated with a resistance-capacitance circuit (time constant 28 ms) and filtered at 0.08 Hz for quantification (*10*, *11*). Integrated RSNA is the area under the curve of RSNA above noise level (*3*, *10–12*). We use the "area under the RSNA curve" as RSNA values. Since both amplitude and "how many fibers are recruited" are reflected

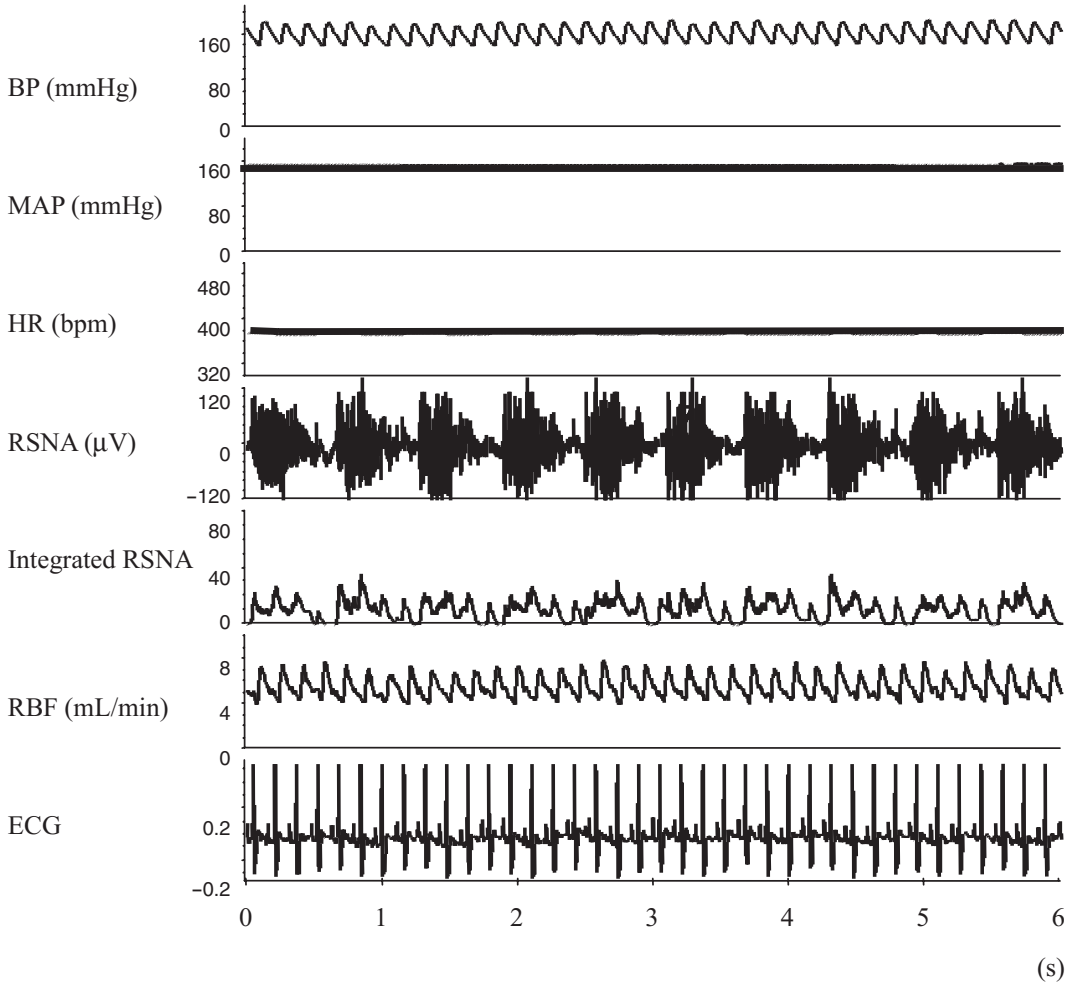

Fig. 1. *An example of actual recordings of the parameters in a conscious vehicle-treated SHR-STZ under unrestrained conditions. Blood pressure (BP), ECG, heart rate (HR), renal sympathetic nerve activity (RSNA), and renal blood flow (RBF) were recorded simultaneously for more than 6.5 min. MAP, mean arterial pressure.*

by the "area under the curve," this technique is more accurate than using only amplitude or frequency of sympathetic nerve signals. The pulsed Doppler probe was connected to a Doppler blood flow velocimeter (PDV-20; CBI Co.). Beat-to-beat BP, ECG, RSNA, and ipsilateral RBF signals were simultaneously recorded in the conscious state for more than 6.5 min. The signals were sampled at 2,000 Hz. An example of actual recordings in an SHR-STZ given vehicle is shown in Fig. 1.

A smoothed instantaneous HR time series was constructed from the R waves of the ECG by using the algorithm proposed by Berger *et al*. (*13*). The time series of BP, HR, RSNA, and RBF was splined and sampled at 64 Hz, so that the values in the entire series were reconstructed to occur simultaneously. In power spectral components of BP, HR, RSNA, and RBF at 0–10 Hz, RSNA peaks were found at frequencies of 0.05, 0.80–0.90, 1–2, and 6–8 Hz. Since RSNA frequencies at 1–2 Hz are known to be respiratory-related and frequencies at 6–8 Hz are cardiac-related, we paid attention to the frequency band below 1.0 Hz. RSNA peaked at 0.05 and 0.80–0.90 Hz; in a previous study we reported tightly coupled rhythmicity between the oscillations of RSNA, BP, HR, and RBF at those frequencies (*3*). Therefore, assuming that RSNA components <1.0 Hz play an important role in regulating BP and RBF, we assessed the linear and nonlinear correlations at the frequency band below 1.0 Hz.

Transfer Function

Linear dependence was quantified by calculating the transfer function from RSNA (as input) to BP or RBF (as output), and from BP (as input) to RBF (as output) (*3*, *14*), and we considered coherence of transfer function as an index of linear correlation. After dividing the data into multiple epochs, we optimized auto- and cross-spectra estimates by ensemble averaging 9–13 data epochs of 4,096 points (64 s) by Welch's method (*1*, *13*). We assessed the significance of coherence

Table 1. Mean Values of Body Weight, Nonfasting Blood Glucose, and Insulin in the 4 Groups

	Body weight (g)	Glucose (mg/dL)	Insulin (ng/mL)
Vehicle (n=10)	245.4±15.1	508.5±40.7	0.25±0.05
Candesartan (n=7)	246.4±17.5	506.3±34.8	0.23±0.07
Amlodipine (n=10)	262.4±17.8	507.5±37.2	0.24±0.06
Insulin (n=10)	328.0±6.9**	190.9±11.2**	3.28±1.22**

**$p<0.01$ *vs*. vehicle. Standard value of fasting rat insulin: 0.5–2 ng/mL. See detail in the text.

values according to Bendat and Piersol (*15*). The details of calculating coherence were previously described by one of the authors of this study (*1*). In the present study we used the same method to assess the significance of coherence values.

Coherence is a measure of the linear statistical link between two variability series at any given frequency. The coherence values of 0–0.5 Hz and 0.5–1.0 Hz were calculated as the maximum values of the respective frequency ranges. This was because RSNA peaked at around 0.05 and 0.80–0.90 Hz and there was tightly coupled rhythmicity between the oscillations of RSNA, BP, HR, and RBF at those frequencies in the previous study (*3*). We considered coherency values >0.5 to be significant (*3*, *15*). The gain between the input signal and the output signal was computed by dividing the ensemble-averaged cross-spectrum by the ensembled power spectrum of the input, and we normalized its magnitude by the ratio between the steady-state value of the output signal and the input signal (*3*). The normalized random error of the coherence function was then estimated at $p<0.05$.

Mutual Information Method

To define the nonlinear and linear relationships between RSNA and BP and between RSNA and RBF, which were passed through a <0.1 Hz low-pass filter, we calculated the mutual information values according to an algorithm proposed by Fraser and Swinney (*4*), as described in our previous studies (*1*, *3*). For each pair of time series, $S = \{s(t)\}$ and $Q = \{q(t)\}$, the mutual information in the system $I(S, Q)$ was defined as the answer to the question "Given a measurement of s, how many bits on average can be predicted for q?"

The mutual information value depends on data length, and the values were normalized to fall between 0 and 1 (*4*). Based on our previous studies (*3*, *5*), mutual information values >0.047 indicate significant linear and nonlinear correlations. The simulation study comparing the original time series with some counterparts contaminated by noise demonstrated that the trend graphs of those time series did not resemble one another in case of mutual information values <0.047. Therefore, if the difference of mutual information between experimental groups is larger than 0.047, it shows that there is a significant difference in hemodynamics (*5*). We calculated the mutual information $I(T)$ of (S, Q), where S is the time series of RSNA(t) and Q is another time series with a time delay T; *e.g.*, S = RSNA(t) and Q = BP($t + T$). Data length was 2^{14} points, *i.e.*, 256 s. T ranged from −5 s to 5 s in 0.25 s steps. I_{max} (S, Q) denotes the maximum value of $I(T)$ between S and Q, when T lies between −5 s and 5 s. We consider the time delay $T_{max}(S, Q)$, at which the maximum value of $I(T)$ of (S, Q) is obtained, to be the physiological delay between these parameters (*5*). When $T_{max}(S, Q)$ is positive, S precedes Q.

The mutual information value gauges the total nonlinear and linear correlation (*4*). Coherence is a measure of the linear statistical link between two variability series at any given frequency. Hence, if a mutual information value in an experimental group is higher than that in the other group and there is no significant difference in coherence between these groups, this indicates that the nonlinear correlation is stronger in the former group than in the latter one.

Protocol

To test the hypothesis that increased RSNA and increased activity of the renin-angiotensin system (RAS) account for the impaired linearity/nonlinearity, we compared linearity and nonlinearity in the vehicle group (n=10), candesartan group (n=7), amlodipine group (n=10), and insulin group (n=10).

Statistics

All values are expressed as means±SEM. Differences were considered significant at $p<0.05$. Differences in BP, HR, RSNA, and RBF parameters were analyzed using the unpaired t-test. Tukey's test for post-hoc multiple comparisons was used to control type 1 error for stricter accuracy.

Results

Basal Values

The mean body weight, nonfasting blood glucose level, and insulin level of each of the 4 groups are shown in Table 1. The blood insulin levels are very low in the vehicle, candesartan, and amlodipine groups, as a result of destruction of islet β cells induced by STZ, which leads to pronounced hyperglycemia and body weight loss. In the insulin group, continuous insulin administration *via* subcutaneous mini-osmotic pumps compensated the lack of insulin, lowered blood glucose level, and prevented loss of body weight. The body weight was higher and the blood glucose level was lower in the insulin group than in the vehicle, candesartan, and amlodipine

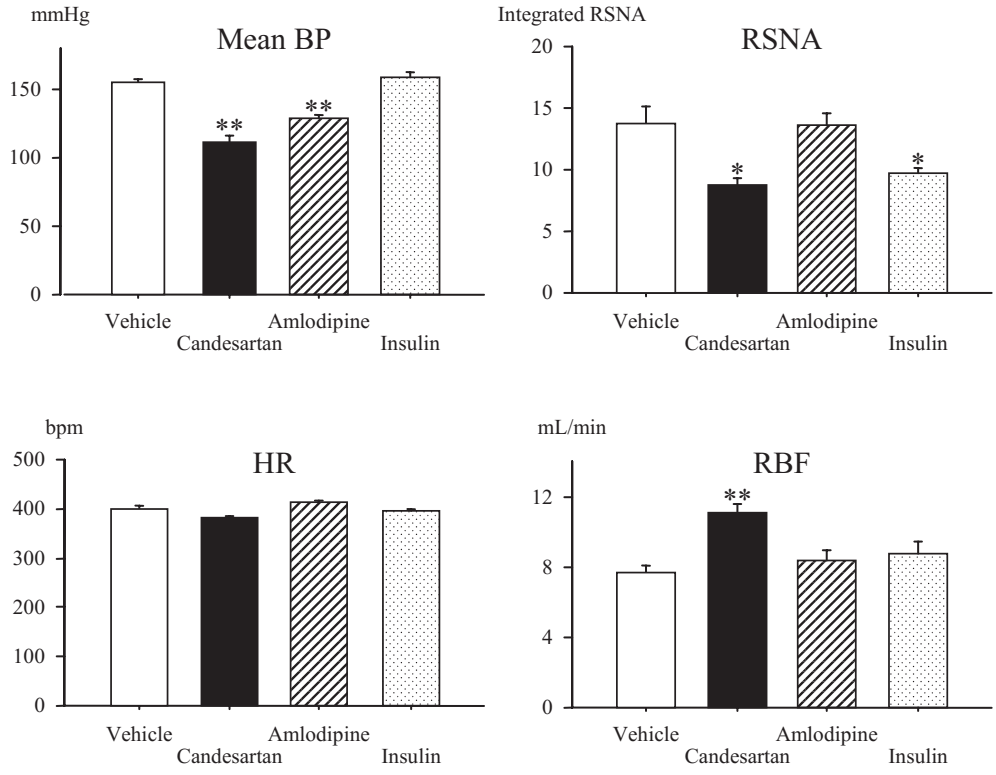

Fig. 2. *Mean values of each parameter obtained in the vehicle group (n = 10), candesartan group (n = 7), amlodipine group (n = 10), and insulin group (n = 10). The mean HR values did not differ significantly between the groups. The mean BP values were significantly lower in the candesartan group and the amlodipine group than in the vehicle group (112 ± 5 and 128 ± 2 mmHg, respectively, vs. 155 ± 3 mmHg, both p < 0.01). The mean RSNA values were significantly lower in the candesartan group and the insulin group than in the vehicle group (8.8 ± 0.6 and 9.7 ± 0.4, respectively, vs. 13.7 ± 1.4, both p < 0.05). Mean RBF values were significantly higher in the candesartan group than in the vehicle group (11.1 ± 0.5 vs. 7.7 ± 0.4 mL/min, p < 0.01). *p < 0.05 vs. vehicle, **p < 0.01 vs. vehicle.*

groups. There were no significant differences in body weight, blood glucose level, or insulin level between the vehicle group, candesartan group, and amlodipine group.

All parameters of HR, BP, RSNA, and RBF among the 4 groups proved to be statistically significant by Tukey's test ($p = 0.0008$, $p < 0.0001$, $p = 0.0016$, $p = 0.0032$, respectively). As shown in Fig. 2, there were no significant differences in mean HR values among the four groups. The BP values were significantly lower in the candesartan group and the amlodipine group than in the vehicle group. The BP values in the candesartan group and amlodipine group were similar. The difference in BP between the insulin group and the vehicle group was not significant. The mean RSNA values in the candesartan and the insulin groups were significantly lower than in the vehicle group (8.8 ± 0.6 and 9.7 ± 0.4, respectively, *vs.* 13.7 ± 1.4, both $p < 0.05$). The RSNA values in the amlodipine group and vehicle group were not significantly different. The mean RBF value was significantly higher in the candesartan group than in the vehicle group (11.1 ± 0.5 *vs.* 7.7 ± 0.4 mL/min, $p < 0.01$). There were no significant differences in RBF values between the amlodipine group, the insulin group, and the vehicle group.

Spectral Analysis

The power spectral components of BP, HR, RSNA, and RBF at 0–10 Hz in the 4 groups revealed RSNA peaks at frequencies around 0–0.10 Hz, 0.80–0.90 Hz, 1–2 Hz, and 6–8 Hz, respectively. An example of the power spectral plots incorporating the mean data of an SHR-STZ given vehicle is shown in Fig. 3. Since RSNA frequencies at 1–2 Hz are known to be respiratory-related, and frequencies at 6–8 Hz are cardiac-related, we focused on the frequency band below 1.0 Hz (*3*).

The power spectra of RSNA peaked at around 0–0.10 and 0.80–0.90 Hz, and as a result of these oscillations in RSNA, a tightly coupled rhythmicity in BP, HR, and RBF was observed at those frequencies. An example of the spectra for coherence of transfer function from RSNA to BP, from BP to RBF, and from RSNA to RBF in the vehicle group is shown in Fig. 4. Assuming that RSNA components < 1.0 Hz play an

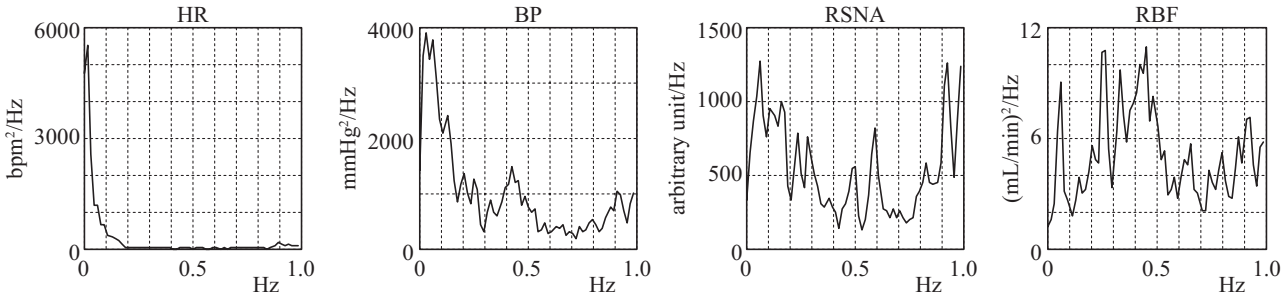

Fig. 3. *An example of the power spectral plots incorporating mean data of a vehicle-treated SHR-STZ.*

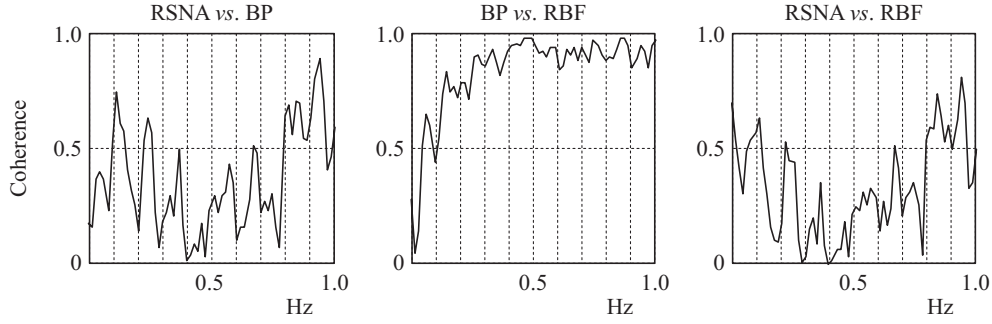

Fig. 4. *An example of spectra for coherence of transfer functions from RSNA to BP, from BP to RBF, and from RSNA to RBF in the vehicle group.*

important role in regulating BP and RBF, we used these data to assess the linear correlation by calculating the transfer function.

Coherence of Transfer Function (Linear Correlation)

Examples of coherence of transfer function from RSNA (as input) to BP (as output) at a frequency band between 0–1.0 Hz in 4 groups are shown in Fig. 5A. Coherence peaked at around 0–0.10 Hz and 0.80–0.90 Hz, showing a significant value of >0.5. As shown in Fig. 5B, there were no significant differences in coherence from RSNA to BP between the four groups.

Similar results were obtained for the transfer function from RSNA (as input) to RBF (as output) and from BP (as input) to RBF (as output) (data not shown). No significant differences in coherence were found between any of the groups.

Mutual Information (Linear and Nonlinear Correlation)

Since they exceeded the value 0.047, the maximum mutual information (I_{max}) values for the correlation between RSNA and BP at 0–1 Hz showed a strong general (both linear and nonlinear) correlation between RSNA and BP (Fig. 6). The I_{max} values were significantly higher in the candesartan group and the insulin group than in the vehicle group (0.34±0.01 and 0.35±0.01, respectively, *vs*. 0.31±0.01, both $p<0.05$), indicating higher nonlinearity in the candesartan group and the insulin group than in the vehicle group. No significant difference in I_{max} values was observed between the amlodipine group and the vehicle group.

Similarly, the significance of the I_{max} values suggested a strong correlation between RSNA and RBF, and the mean value was significantly higher in the candesartan group than in the vehicle group (0.34±0.01 *vs*. 0.31±0.01, $p<0.05$), indicating higher nonlinearity in the candesartan group than in the vehicle group, but no significant difference in I_{max} values was observed between the insulin group and the vehicle group. Any significant difference in I_{max} values was not found between the amlodipine group and the vehicle group (Fig. 6).

Discussion

Effect of Chronic Oral Administration of Candesartan or Amlodipine

Oral administration of candesartan for 2 weeks significantly decreased the RSNA value in hypertensive type 1 diabetic rats despite a significant reduction in BP, whereas amlodipine did not decrease RSNA. ARBs are now recommended as a

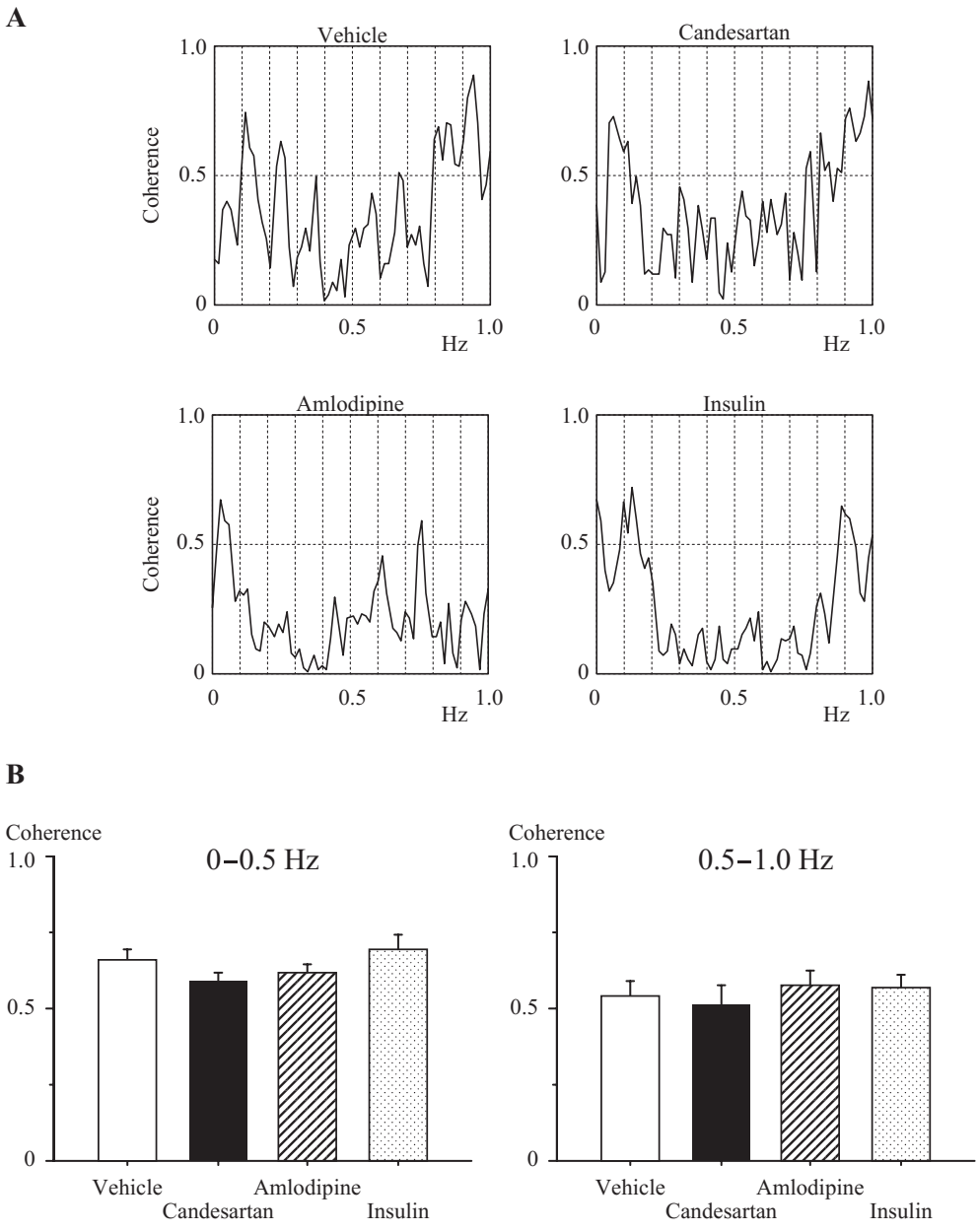

Fig. 5. *Coherence of transfer function from RSNA (as input) to BP (as output). A: In an example of the vehicle group, coherence peaks were found at 0.10 and 0.80–0.90 Hz. In an example of the candesartan group, coherence peaks were found at 0.05 Hz and 0.90–1.0 Hz. In an example of the amlodipine group, coherence peaks were found at 0–0.05 Hz and 0.75 Hz. In an example of the insulin group, coherence peaks were found at 0.1 and 0.9 Hz. Each peak showed a significant value of > 0.5. B: The coherence values of 0–0.5 Hz and 0.5–1.0 Hz in all rats of the each group, calculated as the maximum values of the respective frequency ranges. There were no significant differences in coherence (linear correlation) between RSNA and BP among the groups.*

first line therapy for hypertension associated with diabetes mellitus, and candesartan significantly reduces the risk of cardiovascular death and nonfatal myocardial infarction in patients with heart failure (*16*). We chose candesartan as a representative ARB, since we had demonstrated by the whole-cell patch-clamp technique that it reduces the activity of bulbospinal neurons in the rostral ventrolateral medulla (RVLM, a sympathetic nervous center) of SHR (*17*). Candesartan crossed the blood-brain barrier and reduced the activity of RVLM neurons even when administered orally (*18*). We have also shown that candesartan restores the impaired baroreflex in SHR after myocardial infarction (*12*). Furthermore, candesartan has been reported to produce nitric oxide (NO) and to decrease oxidative stress in animal studies (*19*)

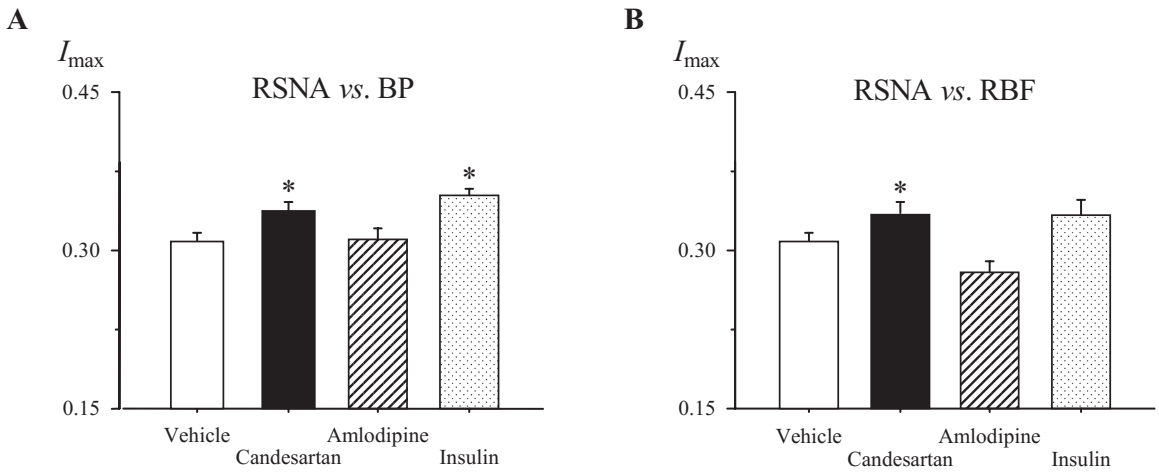

Fig. 6. *Maximum mutual information values (I_{max}) for the correlation between RSNA and BP (A) and between RSNA and RBF (B) at 0–0.1 Hz. The I_{max} values for the correlation between RSNA and BP were higher in the candesartan group and the insulin group than in the vehicle group, indicating higher nonlinearity. No significant differences were found between the I_{max} values in the amlodipine group and vehicle group. The I_{max} values for the correlation between RSNA and RBF were also higher in the candesartan group than in the vehicle group, indicating higher nonlinearity. *$p < 0.05$ vs. vehicle.*

and to decrease carotid intima-media thickness by enhancing NO production and decreasing oxidative stress in patients with hypertension (*20*).

Calcium channel blockers (CCBs) are well known for their strong antihypertensive effect and are widely used clinically. We chose amlodipine because it has beneficial effects against hypertension and hyperglycemia (*21*) and stimulates NO production more than other CCBs (*22*). Amlodipine has been reported to normalize the decreased expression of endothelial nitric oxide synthase gene and protein induced by N^G-nitro-L-arginine methyl ester in hypertensive rats (*23*). NO suppresses the SNS (*24*, *25*), and we previously showed that intravenous infusion of L-arginine, an NO substrate, significantly decreases RSNA and reduces the coherence (linearity) between RSNA and BP in SHR (*3*).

While both candesartan and amlodipine significantly decreased BP to a similar extent in this study, only candesartan reduced RSNA. Candesartan also significantly increased the nonlinear correlations between RSNA and BP and between RSNA and RBF, but neither candesartan nor amlodipine decreased the linear correlation.

The cardiovascular system is regulated by nonlinear dynamics (*26–28*). The nonlinearity of cardiovascular regulation is higher in the healthy state, whereas several diseases, such as congestive heart failure and arrhythmias, are characterized by a reduction in nonlinearity and loss of complexity (*29*). Skinner *et al*. (*30*) and Osaka *et al*. (*31*) have shown that a reduction in the nonlinear correlation of HR intervals preceded ventricular fibrillation or a tachyarrhythmia. The nonlinear correlation of R-R intervals is a better predictor of risk than a stochastic measure such as the standard deviation. Huikuri *et al*. found a reduction in the nonlinear correlation of HR intervals to be an independent robust predictor of mortality after an acute myocardial infarction (*32*). They showed that the short-term fractal scaling exponent (nonlinear analysis) to be the most powerful predictor of death compared with other HR variability measures (linear analysis) in patients with depressed left ventricular function after an acute myocardial infarction (*32*). Complex fluctuations with the statistical properties of fractals have not only been described for HR variability but also for fluctuations in systemic BP (*33*).

Reduced nonlinearity of HR variability is an independent predictor of high mortality in patients who have had a myocardial infarction (*32*), and the results of our study suggest that candesartan improved the nonlinearity in conscious hypertensive type 1 diabetic rats. The effect of lowering BP itself may have been a factor in improving the nonlinear correlations. However, while candesartan and amlodipine reduced BP to the same extent, only candesartan improved the nonlinearity in SHR-STZ.

We think that the higher nonlinearity in the candesartan group compared with the vehicle group (Fig. 6) can be explained by the fact that BP and RBF are regulated by many neurohumoral elements, not only by RSNA or RAS. As Goldberger (*29*) stated, nonlinearity is high in the normal cardiovascular system, since many elements, such as baroreflexes and NO, are involved. In contrast, in certain diseases, such as congestive heart failure, arrhythmias, and epilepsy, nonlinearity is low and complexity is lost (*29*). In the present study RSNA was significantly reduced in the candesartan group but not in the amlodipine group, and in a previous study we showed that candesartan reduces the activity of RVLM neurons in SHR (*17*). We have also demonstrated that candesartan improves impaired baroreflex in conscious SHR (*12*).

The baroreflex system contributes to high nonlinearity and complexity, because nonlinearity is decreased and the SNS is activated in baroreceptor-denervated normotensive dogs (*28*). Finally, the candesartan-induced increase in NO production may contribute to the improvement in nonlinearity, because 3-d intravenous administration of candesartan increased NO production in type 1 diabetic mice (*34*).

In contrast, the lower nonlinearity in the vehicle-treated diabetic rats (Fig. 6) was probably due to the cardiovascular system strongly depending on only one or two predominant neurohumoral elements (the SNS and RAS) and/or due to the fact that the number of neurohumoral elements is decreased (*29*). Thus, by simultaneously recording BP, HR, RSNA, and RBF in the conscious state, we were able to show that the increased RSNA plays a key role in reducing the nonlinearity in hypertensive type 1 diabetes.

Effect of Chronic Insulin Treatment

A number of previous studies have reported that acute hyperinsulinemic euglycemic clamping of healthy subjects induces sympathetic activation, measured as increased plasma norepinephrine levels or augmented muscle sympathetic nerve activity (MSNA) (*9*, *35*). However, Gans *et al*. (*36*) found no significant changes in plasma catecholamine levels during the insulin clamp, and neither sympathetic nerve activity nor BP is elevated in insulinoma patients, who have fasting insulin concentrations four- to five-fold higher than healthy subjects (*37*). Esler and colleagues (*38*, *39*) have clearly demonstrated that serum insulin concentration and renal noradrenaline spillover are not quantitatively related at all, arguing against hyperinsulinemia stimulating the SNS. Thus, the increase in sympathetic nerve activity from acute insulin infusion observed in the past studies (*9*, *35*) may result from baroreflex-mediated adjustments to the systemic vasodilatation.

In the present study we subcutaneously administered insulin for 2 weeks to rats with type 1 diabetes. Chronic administration of rapid insulin *via* subcutaneous mini-osmotic pumps significantly lowered blood glucose and decreased the values of integrated RSNA. We recognize the underlying difference in duration between acute insulin infusion and our 2-week infusion study. Another difference is that we gave insulin to type 1 diabetic rats instead of to healthy humans (*9*, *35*), thereby reducing their high blood glucose level to an almost normal state, as shown in Table 1. Although the impact of these differences cannot be excluded and the effect of diabetes may be a contributing factor for the change, we have shown a significant decrease in RSNA and a significant increase in nonlinearity between RSNA and BP without altering BP as a result of chronic insulin administration. If the relationship between RSNA and BP is totally linear, then BP should fall as RSNA decreases. But since their relationship is nonlinear, many factors are involved in the regulation of BP. Chronic insulin administration increased the nonlinearity between BP and RSNA, making BP depend not only on RSNA, but also on many other factors such as endothelial function, advanced glycation end products (AGE), NO, and baroreflex, which make the neuro-vascular system more complex. Thus, insulin, which lowers the blood glucose concentration in type 1 diabetes, may be beneficial in improving the autonomic nervous system dysfunction and neurohumoral regulation of the cardiovascular system.

We designed this study in type 1 diabetic hypertensive rats to investigate the changes in BP, HR, RSNA, RBF, and nonlinear correlations of cardiovascular regulation after administering drugs. The results showed that candesartan, but not amlodipine, decreased RSNA and increased the nonlinearity of correlation between BP and RSNA, despite significantly lowering BP. Candesartan also significantly increased RBF, probably due to the blockade of angiotensin II actions. These results suggest that reducing high sympathetic nerve activity and the activity of the RAS may be important to increasing nonlinearity, which in turn may lead to a more healthy state (*32*). It would be interesting to measure RAS activity in subsequent studies. In this study, chronic insulin administration decreased RSNA and increased nonlinearity without altering BP. Alternatively, lowering high blood glucose itself may be a contributing factor, and further study is needed to determine whether this is true. Considering the fact that 95% of diabetic patients have type 2 diabetes, the next study should be conducted on rats with type 2 diabetes.

In summary, although both candesartan and amlodipine lowered BP similarly, only candesartan decreased RSNA and increased nonlinearity of correlation between RSNA and BP. Chronic insulin administration that lowered blood glucose level decreased RSNA and increased nonlinearity without affecting BP. These results suggest that reducing RSNA and the activity of the RAS may be important in increasing the nonlinearity of cardiovascular regulation in diabetic hypertension.

Limitations of This Study

A limitation of this study is the reliability of interpretation of multifiber RSNA recordings between rats in order to assess SNA, related to issues concerning variations in numbers of fibers or variation in nerve and electrode contact. We are using the “area under the curve” of RSNA curves as RSNA values. Since both amplitude and number of fibers recruited are reflected in the “area under the curve” method, it should be more accurate than using only amplitude or frequency of RSNA. It is also likely to be more accurate than presenting human MSNA by burst/min or burst/100 heartbeats. Calibrating each preparation by using nasopharyngeal stimulation is difficult in conscious rats because of the technique’s limited characterization. Measuring whole body norepinephrine spill over or plasma norepinephrine levels is another alternative for representing SNA. However, tissue or plasma norepinephrine levels alone are not sufficient to represent RSNA, since they vary between rats and depend on a number of factors such as

rate of synthesis, uptake, and release of norepinephrine by the tissues. Applying them together with other methods to assess RSNA may be the most desirable approach in future studies.

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