Original Article

Effects of a New Calcium Channel Blocker, Azelnidipine, on Systemic Hemodynamics and Renal Sympathetic Nerve Activity in Spontaneously Hypertensive Rats

Takatomi SHOKOJI*, Yoshihide FUJISAWA**, Hideyasu KIYOMOTO***, Matlubur RAHMAN*,***, Guang-Ping SUN***, Yu-Yan FAN*, Shoji KIMURA*, Masakazu KOHNO***, Youichi ABE*, and Akira NISHIYAMA*

Antihypertensive treatment with dihydropyridine calcium channel blockers elicits sympathetic nerve activation, which may contribute to cardiovascular events. However, recent clinical studies showed that treatment with azelnidipine, a new dihydropyridine calcium channel blocker, significantly reduced blood pressure in hypertensive patients while either maintaining or actually decreasing heart rate (HR). In this study, we examined the effects of azelnidipine and amlodipine on systemic hemodynamics and renal sympathetic nerve activity (RSNA) in anesthetized spontaneously hypertensive rats (SHR). We also examined the effects of these agents on baroreflex functions by infusing phenylephrine (30 μg/kg/min, i.v.) and sodium nitroprusside (10 μg/kg/min, i.v.) into azelnidipine- or amlodipine-treated SHR. Fifty min after administration of azelnidipine (10 μg/kg/min for 10 min, i.v.), mean arterial pressure (MAP) significantly decreased from 153±5 to 122±5 mmHg; however, HR and integrated RSNA did not change significantly (from 352±9 to 353±10 beats/min and 115±5% of baseline, respectively). Infusion of amlodipine (50 μg/kg/min for 10 min) elicited similar effects on MAP (from 152±5 to 120±4 mmHg). However, amlodipine significantly increased HR (from 351±9 to 375±11 beats/min) and integrated RSNA (165±5% of baseline). Analyses of baroreflex function curves revealed that azelnidipine-treated rats showed a smaller baroreflex function than amlodipine-treated rats ($p<0.05$). These data suggest that azelnidipine possesses sympathoinhibitory effects, which may be one reason why it had less pronounced effects on HR in hypertensive patients. (*Hypertens Res* 2005; 28: 1017–1023)

***Key Words*: azelnidipine, amlodipine, renal sympathetic nerve activity (RSNA), spontaneously hypertensive rats (SHR), heart rate (HR)**

From the *Department of Pharmacology, **Research Equipment Center, and ***Second Department of Internal Medicine, Kagawa University Medical School, Kagawa, Japan.

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (17790171), by a Salt Sciences Research Grant (05C2), and by a grant from the Japan Research Foundation for Clinical Pharmacology (to A.N.).

Address for Reprints: Akira Nishiyama, M.D., Ph.D., Department of Pharmacology, Kagawa University Medical School, 1750–1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761–0793, Japan. E-mail: akira@kms.ac.jp

Received August 15, 2005; Accepted in revised form October 17, 2005.

Introduction

Dihydropyridine calcium channel blockers (CCBs) have potent antihypertensive effects with few adverse reactions and are the most common antihypertensive drugs prescribed in Japan (*1*). However, it has been revealed that nifedipine, a short-acting dihydropyridine CCB, increases the risks of ischemic heart disease (*2*, *3*), a phenomenon that is thought to be mainly related to its reflex responses, *i.e.*, activation of the sympathetic nervous system and tachycardia after rapid lowering of blood pressure (*4*). Therefore, the use of long-acting dihydropyridine CCBs is generally recommended in hypertensive patients (*5*, *6*). Nevertheless, clinical studies in hypertensive patients have reported inconsistent data regarding the responses of sympathetic nerve activity and norepinephrine (NE) release to conventional long-acting CCBs, such as coat-core nifedipine or amlodipine (*7–13*). While some studies showed that coat-core nifedipine or amlodipine did not affect sympathetic nerve activity or plasma NE levels (*7–11*), other studies reported increased effects during antihypertensive treatment with these drugs (*10–13*).

Azelnidipine is a new dihydropyridine CCB that is highly lipid soluble and has prolonged antihypertensive properties (its plasma half life is about 15–21 h) (*14*). Arita *et al*. (*15*) reported that antihypertensive treatment with azelnidipine for 4 weeks did not affect heart rate (HR) or plasma levels of epinephrine and NE despite significant blood pressure reduction. Recently, Kuramoto *et al*. (*13*) examined effects of azelnidipine and amlodipine on blood pressure and HR using 24-h monitoring with a portable automatic monitor system in patients with essential hypertension. The authors observed that both drugs had a similar stable antihypertensive effect lasting for 24 h following administration. Of interest, although amlodipine increased HR, azelnidipine significantly decreased HR. These observations suggest that azelnidipine possesses inhibitory effects on the autonomic system. However, to date, the response of sympathetic nerve activity to azelnidipine treatment has not been investigated.

In the present study, we examined the effects of azelnidipine on renal sympathetic nerve activity (RSNA) in spontaneously hypertensive rats (SHR). In addition, we compared the effects of azelnidipine on RSNA with those of amlodipine and sodium nitroprusside. Finally, we examined the effects of azelnidipine and amlodipine on baroreflex functions by analyzing baroreflex function curves (*16*).

Methods

Animal Preparation

Experiments were performed in 13- to 14-week-old male SHR (Charles River Japan, Inc., Yokohama, Japan). All surgical and experimental procedures were performed according to the guidelines and practices established by the Animal Care and Use Committee of Kagawa University Medical School. The surgical preparation of animals and basic experimental techniques were identical to those previously described (*16–18*). Briefly, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and given additional doses as required. A polyethylene catheter was inserted into the abdominal aorta *via* the right femoral artery, and MAP and HR were continuously monitored using a pressure transducer (model 361; NEC–San-ei, Tokyo, Japan). A catheter was also inserted into the inferior vena cava *via* the right femoral vein, and an isotonic saline solution was infused at a rate of 2 ml/h for the duration of stabilization and experiments. The left kidney was exposed through a retroperitoneal flank incision. The renal nerve branch was then isolated near the aortic-renal arterial junction and placed on a Teflon-coated stainless steel bipolar electrode. Thereafter, the renal nerve and electrode were covered with silicone rubber. Renal nerve discharge was amplified using a differential amplifier (AVB-10; Nihon Kohden, Tokyo, Japan) with a band-pass filter. Amplified and filtered signals were visualized on a dual-beam oscilloscope (VC-10; Nihon Kohden), and monitored by an audio speaker. Output from the amplifier was integrated by an integrator (model 1322; NEC–San-ei) with a 1-s resetting. Changes in nerve activity were expressed as percentages of the control resting spontaneous nerve activity.

Experimental Protocols

Effects of Azelnidipine, Amlodipine and Sodium Nitroprusside on Systemic Hemodynamics and RSNA

After a 60 min stabilization period following completion of surgery, the experimental protocol was started by recording basal MAP, HR and RSNA. Then, azelnidipine (Sankyo Co., Ltd., Tokyo, Japan) was intravenously administered for 10 min (10 μg/kg/min, $n=10$). After cessation of azelnidipine infusion, MAP, HR and RSNA were continuously monitored for more than 110 min. In a separate group of animals, amlodipine (50 μg/kg/min, $n=10$) or sodium nitroprusside (3 μg/kg/min, $n=10$) was infused intravenously for 10 min, and MAP, HR and RSNA were continuously monitored in a similar manner as in the azelnidipine group. Azelnidipine and amlodipine were dissolved in dimethylformamide with an isotonic saline solution (less than 0.1%, respectively), as described previously (*19*). Sodium nitroprusside was dissolved in an isotonic saline solution. Preliminary experiments showed that infusion of dimethylformamide or saline solution did not alter MAP, HR or RSNA ($n=3$, respectively, data not shown). Furthermore, we found that infusion of azelnidipine (10 μg/kg/min) and amlodipine (50 μg/kg/min) for 10 min decreased MAP to a similar extent in SHR ($n=3$, respectively, data not shown). Doses of amlodipine and sodium nitroprusside were determined on the basis of results from previous studies on rats (*16*, *20*).

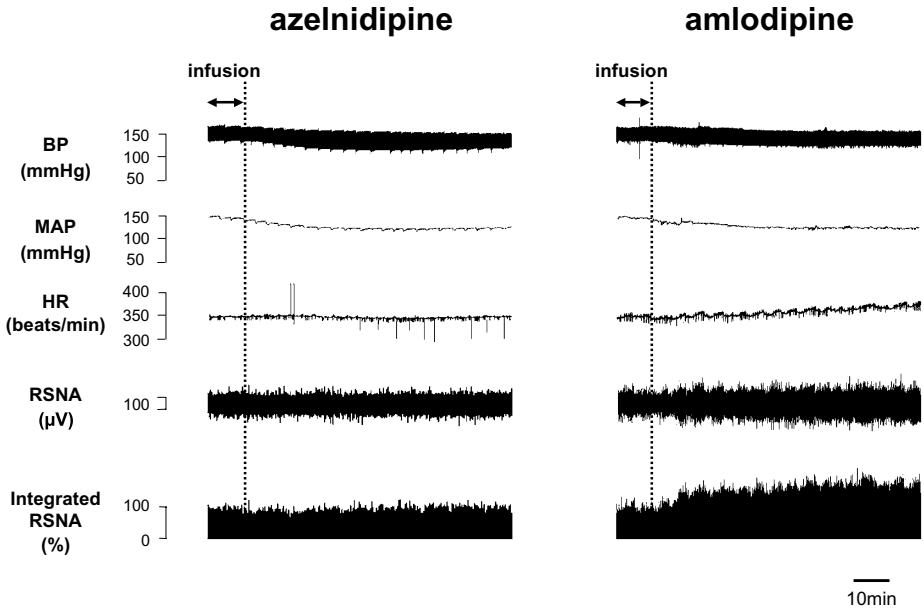

Fig. 1. *Typical responses of blood pressure (BP), mean arterial pressure (MAP), heart rate (HR), renal sympathetic nerve activity (RSNA), and integrated RSNA to azelnidipine and amlodipine in spontaneously hypertensive rats (SHR). Azelnidipine and amlodipine were dissolved in dimethylformamide with an isotonic saline solution (less than 0.1%, respectively).*

Effects of Phenylephrine or Sodium Nitroprusside on Systemic Hemodynamics and RSNA after Treatment with Azelnidipine or Amlodipine

After recording basal MAP, HR and RSNA, azelnidipine (10 μg/kg/min, $n=10$) was infused for 10 min, as described above. Ninety min after the end of azelnidipine infusion, phenylephrine (30 μg/kg/min, $n=5$) or sodium nitroprusside (10 μg/kg/min, $n=5$) was infused intravenously, and MAP, HR and RSNA were continuously monitored. In a separate group of animals, amlodipine (50 μg/kg/min, $n=10$) was infused intravenously for 10 min in a similar manner as azelnidipine. Ninety min after cessation of amlodipine infusion, phenylephrine (30 μg/kg/min, $n=5$) or sodium nitroprusside (10 μg/kg/min, $n=5$) was administered. Both phenylephrine and sodium nitroprusside were dissolved in isotonic saline solution. In preliminary experiments, we observed that the response of MAP to azelnidipine and amlodipine peaked at 60 min after infusion, and remained stable for more than 60 min ($n=4$, respectively, data not shown). Doses of sodium nitroprusside and phenylephrine were determined on the basis of results from previous studies on rats (*16*).

Statistical Analysis

Values are presented as the means±SEM. Statistical comparisons of differences were performed using a one-way or two-way analysis of variance for repeated measures combined with a Newman-Keuls post hoc test. Values of $p<0.05$ were considered to indicate statistical significance.

Results

Responses of Systemic Hemodynamics and RSNA to Azelnidipine, Amlodipine or Sodium Nitroprusside

Typical responses of systemic hemodynamics and RSNA to administration of azelnidipine and amlodipine are shown in Fig. 1. Intravenous infusion of azelnidipine and amlodipine for 10 min significantly decreased MAP from 153±5 to 138±6 mmHg and from 152±5 to 140±5 mmHg, respectively. On the other hand, significant changes in HR and RSNA were not observed during infusion of azelnidipine or amlodipine (Fig. 2). Intravenous infusion of sodium nitroprusside rapidly decreased MAP by 29±4 mmHg and increased HR and RSNA by 42±5 beats/min and 135±5%, respectively (at 10 min infusion for each, Fig. 2).

After cessation of azelnidipine or amlodipine infusions, MAP continued to decrease (Fig. 2). The azelnidipine- and amlodipine-induced MAP reductions peaked at about 50 min after infusion cessation (122±5 and 120±4 mmHg, respectively). In azelnidipine-treated animals, significant changes in HR and integrated RSNA were not observed during the experimental period. However, HR and RSNA were significantly increased in amlodipine-treated animals (by 24±3 beats/min and 65±5% at 50 min after end of infusion, respectively, Fig. 2). On the other hand, sodium nitroprusside-induced changes in MAP, HR and RSNA returned to the control levels immediately after cessation of infusion (Fig. 2).

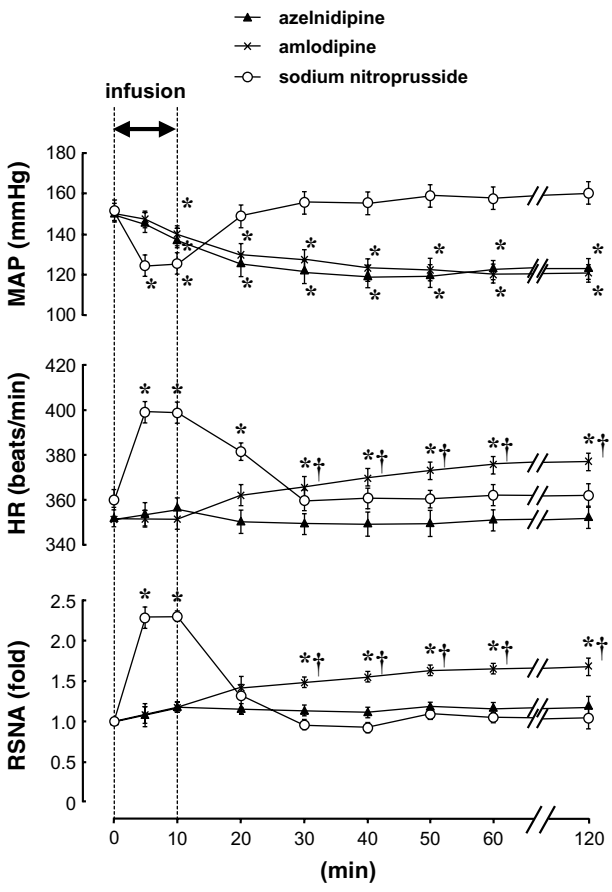

Fig. 2. *Effects of intravenous infusions of azelnidipine, amlodipine or sodium nitroprusside on MAP, HR and integrated RSNA in SHR. Azelnidipine or amlodipine infusion for 10 min gradually decreased MAP, but did not change HR and RSNA. On the other hand, sodium nitroprusside rapidly decreased MAP and increased HR and RSNA. After cessation of azelnidipine or amlodipine infusion, MAP decreased further. In azelnidipine-treated animals, significant changes in HR and integrated RSNA were not observed during the experimental period. However, HR and RSNA significantly increased in amlodipine-treated animals. On the other hand, the sodium nitroprusside-induced decrease in MAP and increases in HR and RSNA returned to the control values immediately after cessation of infusion.* *$p < 0.05$ vs. *baseline (0 min).* †$p < 0.05$: *azelnidipine* vs. *amlodipine.*

Responses of Systemic Hemodynamics and RSNA to Phenylephrine and Sodium Nitroprusside after Treatment with Azelnidipine or Amlodipine

The effects of azelnidipine on baroreflex functions were examined by infusing phenylephrine and sodium nitroprusside, as described previously (*16*). Ninety min after the end of azelnidipine infusion, MAP was 120±3 mmHg. Intravenous infusion of phenylephrine significantly increased MAP and decreased RSNA. When phenylephrine-induced increases in MAP reached 20 and 40 mmHg in azelnidipine-treated SHR, RSNA decreased by 10±3% and 17±3%, respectively (Fig. 3). On the other hand, sodium nitroprusside significantly decreased MAP and increased RSNA in azelnidipine-treated SHR. When sodium nitroprusside decreased MAP by 20 and 40 mmHg, RSNA was increased by 5±2% and 6±1%, respectively (Fig. 3). In amlodipine-treated SHR, basal MAP was similar to that in azelnidipine-treated animals (119±3 mmHg). When the phenylephrine-induced increases in MAP reached 20 and 40 mmHg in amlodipine-treated SHR, RSNA was decreased by 11±5% and 39±4%, respectively (Fig. 3). On the other hand, when sodium nitroprusside decreased MAP by 20 and 40 mmHg, RSNA was increased by 9±2% and 17±4%, respectively (Fig. 3). Based on group comparisons, the magnitude of the phenylephrine-induced reduction in RSNA in amlodipine-treated animals was significantly greater than that in azelnidipine-treated animals ($p<0.05$, Fig. 3). Similarly, the magnitude of the nitroprusside-induced increase in RSNA in amlodipine-treated animals was significantly greater than that in azelnidipine-treated animals ($p<0.05$, Fig. 3).

Discussion

Dihydropyridine CCBs have been shown to cause reflex sympathetic stimulation and tachycardia in association with a decrease in blood pressure (*4*, *21*), and this increased cardiac workload has been considered responsible for their limited preventive effects against cardiac complications (*2*, *3*). However, recent clinical studies indicated that tachycardia was not associated with the antihypertensive effects of azelnidipine, a new lipophilic dihydropyridine CCB (*13*, *15*). The present study provided the first evidence that azelnidipine had less pronounced effects on sympathetic nerve activity in SHR compared to other CCBs, despite a significant blood pressure reduction. These data along with those of recent clinical studies (*13*, *15*) indicate that azelnidipine avoids the disadvantages of conventional CCBs, including adverse effects on the heart, and might, therefore, be beneficial for treating hypertensive patients.

The slow and prolonged antihypertensive action of long-acting CCBs might be responsible for their inhibitory effects on reflex nerve stimulation (*22*). In the present study, we compared the effects of another long-acting dihydropyridine CCB, amlodipine, on HR and RSNA with those of azelnidipine. Several clinical studies previously reported that antihypertensive treatment with amlodipine had no effects on either sympathetic nerve activity or HR (*7–10*). However, it has also been shown that amlodipine treatment significantly increased HR and plasma NE levels (*11–13*). The reasons for these discrepancies among clinical observations are not clear; however, they may be due to differences in the experimental settings, including the patient populations, the patient back-

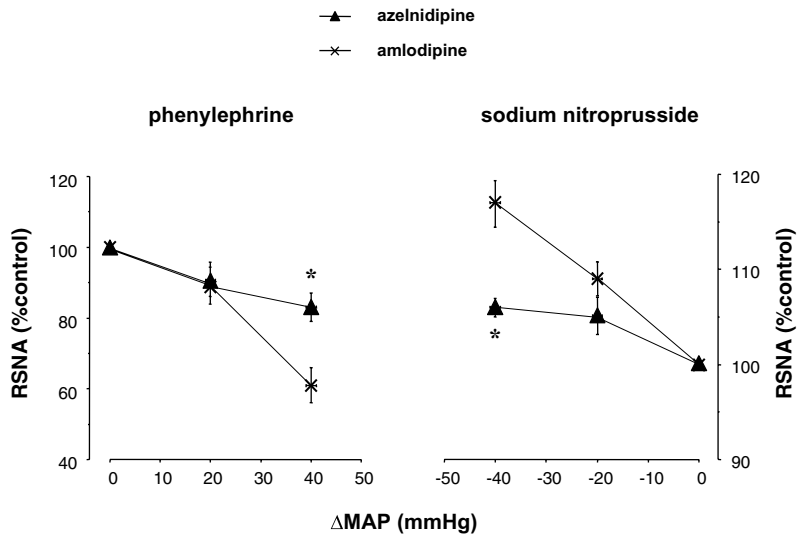

Fig. 3. *Effects of azelnidipine and amlodipine on baroreflex functions were examined by infusion of phenylephrine and sodium nitroprusside. The magnitude of the phenylephrine-induced reduction in RSNA in amlodipine-treated SHR was significantly greater than that in azelnidipine-treated SHR. Similarly, the magnitude of the nitroprusside-induced increase in RSNA in amlodipine-treated SHR was significantly greater than that in azelnidipine-SHR.* *$p < 0.05$*: azelnidipine* vs. *amlodipine.*

grounds, and the durations or doses of amlodipine treatment. In the present study, we observed that in anesthetized SHR, systemic administration of amlodipine resulted in a similar slow and prolonged blood pressure reduction in response to azelnidipine. However, amlodipine significantly increased HR and RSNA, whereas azelnidipine did not affect these parameters. Thus, azelnidipine showed lesser effects on sympathetic nerve activity, which might not be simply explained by its slow and prolonged antihypertensive action.

Dihydropyridine CCBs have high binding affinity to L-type calcium channels (*23*). However, it has also been reported that some dihydropyridine CCBs, including amlodipine, blocked N-type and T-type calcium channels (*24–26*), which might then suppress sympathetic stimulation and atrial conduction, respectively (*23*). Koike *et al*. (*14*) used a patch-clamp technique and examined effects of azelnidipine on N-type and T-type calcium channels in PC12 cells. The authors showed that azelnidipine minimally affected N-type and T-type calcium channels (*14*). In the present study, we examined the effects of these agents on baroreflex functions by infusing phenylephrine and sodium nitroprusside. We observed that phenylephrine- or nitroprusside-induced changes in blood pressure were similar in azelnidipine- and amlodipine-treated SHR. However, the magnitudes of the phenylephrine-induced reductions in HR and RSNA in amlodipine-treated SHR were significantly greater than those in azelnidipine-treated SHR. Similarly, the magnitudes of sodium nitroprusside-induced increases in HR and RSNA in amlodipine-treated SHR were significantly greater than those in azelnidipine-treated SHR. These data suggest that the sympathoinhibitory effects of azelnidipine are mediated, at least in part, through inhibition of baroreflex functions. However, the precise mechanisms by which azelnidipine exerts its sympathoinhibitory effects on baroreflex functions are still not clear.

A growing body of evidence suggests that reactive oxygen species (ROS) are involved in the regulation of sympathetic nerve activity (*17*, *18*, *27*, *28*). We previously showed that systemic administration of the superoxide dismutase (SOD) mimetic, tempol, resulted in decreases in MAP and HR along with a reduction in RSNA (*17*, *18*). We also showed that these parameters were significantly increased by systemic administration of the SOD inhibitor, diethyldithio-carbamic (DETC). Gao *et al*. (*27*) showed that intracerebroventricular administration of tempol or the NADPH oxidase inhibitor, apocynin, decreased RSNA in rabbits with chronic heart failure. In the same study, they showed that intracerebroventricular administration of DETC significantly increased RSNA in these animals (*27*). These observations suggest that ROS serve as important regulators of the sympathetic nervous system. Interestingly, Shinomiya *et al*. (*29*) showed that azelnidipine exhibited a stronger anti-oxidative activity in human endothelial cells than amlodipine or nifedipine. Similarly, azelnidipine inhibited tumor necrosis factor-α–induced NADPH oxidase activation in endothelial cells (*30*). Therefore, it is interesting to speculate that some of the sympathoinhibitory effects of azelnidipine are mediated *via* its antioxidative activity. Obviously, however, further studies will be needed to address these issues.

In conclusion, the results in the present study suggest that azelnidipine elicits sympathoinhibitory effects through a reduction in baroreflex functions, which might be one reason

for its less pronounced effects on HR in hypertensive patients compared to other CCBs. It is possible that azelnidipine avoids the disadvantages of conventional CCBs, including their adverse effects on the heart, and may, therefore, be beneficial for treating hypertensive patients. However, further studies will be needed to investigate the likelihood of adverse reactions, including orthostatic hypotension, in azelnidipine-treated hypertensive patients.

Acknowledgements

We are grateful to Sankyo, Co., Ltd. (Tokyo, Japan) for supplying azelnidipine.

References

1. Ogihara T, Hiwada K, Morimoto S, *et al*: Guidelines for treatment of hypertension in the elderly—2002 revised version. *Hypertens Res* 2003; **26**: 1–36.
2. Psaty BM, Heckbert SR, Koepsell TD, *et al*: The risk of myocardial infarction associated with antihypertensive drug therapies. *JAMA* 1995; **274**: 620–625.
3. Furberg CD, Psaty BM, Meyer JV: Nifedipine. Dose-related increase in mortality in patients with coronary heart disease. *Circulation* 1995; **92**: 1326–1331.
4. Leenen FH, Ruzicka M, Huang BS: Central sympathoinhibitory effects of calcium channel blockers. *Curr Hypertens Rep* 2001; **3**: 314–321.
5. Yui Y, Sumiyoshi T, Kodama K, *et al*, Japan Multicenter Investigation for Cardiovascular Diseases-B Study Group: Comparison of nifedipine retard with angiotensin converting enzyme inhibitors in Japanese hypertensive patients with coronary artery disease: the Japan Multicenter Investigation for Cardiovascular Diseases-B (JMIC-B) randomized trial. *Hypertens Res* 2004; **27**: 181–191.
6. Iino Y, Hayashi M, Kawamura T, *et al*, Japanese Losartan Therapy Intended for the Global Renal Protection in Hypertensive Patients (JLIGHT) Study Investigators: Renoprotective effect of losartan in comparison to amlodipine in patients with chronic kidney disease and hypertension—a report of the Japanese Losartan Therapy Intended for the Global Renal Protection in Hypertensive Patients (JLIGHT) study. *Hypertens Res* 2004; **27**: 21–30.
7. Minami J, Ishimitsu T, Kawano Y, Matsuoka H: Effects of amlodipine and nifedipine retard on autonomic nerve activity in hypertensive patients. *Clin Exp Pharmacol Physiol* 1998; **25**: 572–576.
8. Grassi G, Spaziani D, Seravalle G, *et al*: Effects of amlodipine on sympathetic nerve traffic and baroreflex control of circulation in heart failure. *Hypertension* 1999; **33**: 671–675.
9. Binggeli C, Corti R, Sudano I, Luscher TF, Noll G: Effects of chronic calcium channel blockade on sympathetic nerve activity in hypertension. *Hypertension* 2002; **39**: 892–896.
10. Hamada T, Watanabe M, Kaneda T, *et al*: Evaluation of changes in sympathetic nerve activity and heart rate in essential hypertensive patients induced by amlodipine and nifedipine. *J Hypertens* 1998; **16**: 111–118.
11. Tsutamoto T, Tsutsui T, Maeda K, *et al*: Effects of long-acting calcium channel antagonists on neurohumoral factors: comparison of nifedipine coat-core with amlodipine. *J Cardiovasc Pharmacol* 2003; **41**: S77–S81.
12. Eguchi K, Kario K, Shimada K: Differential effects of a long-acting angiotensin converting enzyme inhibitor (temocapril) and a long-acting calcium antagonist (amlodipine) on ventricular ectopic beats in older hypertensive patients. *Hypertens Res* 2002; **25**: 329–333.
13. Kuramoto K, Ichikawa S, Hirai A, Kanada S, Nakachi T, Ogihara T: Azelnidipine and amlodipine: a comparison of their pharmacokinetics and effects on ambulatory blood pressure. *Hypertens Res* 2003; **26**: 201–208.
14. Koike H, Kimura T, Kawasaki T, *et al*: Azelnidipine, a long-acting calcium channel blocker with slow onset and high vascular affinity. *Annu Rep Sankyo Res Lab* 2002; **54**: 1–64.
15. Arita M, Hashizume T, Tanigawa K, Yamamoto H, Nishio I: A new Ca-antagonist, azelnidipine, reduced blood pressure during exercise without augmentation of sympathetic nervous system in essential hypertension: a randomized, double-blind, placebo-controlled trial. *J Cardiovasc Pharmacol* 1999; **33**: 186–192.
16. Fujisawa Y, Mori N, Yube K, Miyanaka H, Miyatake A, Abe Y: Role of nitric oxide in regulation of renal sympathetic nerve activity during hemorrhage in conscious rats. *Am J Physiol* 1999; **277**: H8–H14.
17. Shokoji T, Nishiyama A, Fujisawa Y, *et al*: Renal sympathetic nerve responses to tempol in spontaneously hypertensive rats. *Hypertension* 2003; **41**: 266–273.
18. Shokoji T, Fujisawa Y, Kimura S, *et al*: Effects of local administrations of tempol and diethyldithio-carbamic on peripheral nerve activity. *Hypertension* 2004; **44**: 236–243.
19. Oizumi K, Nishino H, Koike H, Sada T, Miyamoto M, Kimura T: Antihypertensive effects of CS-905, a novel dihydropyridine Ca^{++} channel blocker. *Jpn J Pharmacol* 1989; **51**: 57–64.
20. Huang BS, Leenen FH: Sympathoinhibitory and depressor effects of amlodipine in spontaneously hypertensive rats. *J Cardiovasc Pharmacol* 2003; **42**: 153–160.
21. Frohlich ED, McLoughlin MJ, Losem CJ, Ketelhut R, Messerli FH: Hemodynamic comparison of two nifedipine formulations in patients with essential hypertension. *Am J Cardiol* 1991; **68**: 1346–1350.
22. Noll G, Wenzel RR, Shaw S, Luscher TF: Calcium antagonists and sympathetic nerve activation: are there differences between classes? *J Hypertens Suppl* 1998; **16**: S17–S24.
23. Varadi G, Mori Y, Mikala G, Schwartz A: Molecular determinants of Ca^{2+} channel function and drug action. *Trends Pharmacol Sci* 1995; **16**: 43–49.
24. Furukawa T, Yamakawa T, Midera T, Sagawa T, Mori Y, Nukada T: Selectivities of dihydropyridine derivatives in blocking Ca^{2+} channel subtypes expressed in Xenopus oocytes. *J Pharmacol Exp Ther* 1999; **291**: 464–473.
25. Furukawa T, Nukada T, Miura R, *et al*: Differential blocking action of dihydropyridine Ca^{2+} antagonists on a T-type Ca^{2+} channel (alpha1G) expressed in Xenopus oocytes. *J Cardiovasc Pharmacol* 2005; **45**: 241–246.
26. Takahara A, Fujita S, Moki K, *et al*: Neuronal Ca^{2+} channel blocking action of an antihypertensive drug, cilnidipine, in IMR-32 human neuroblastoma cells. *Hypertens Res* 2003;

26: 743–747.
27. Gao L, Wang W, Li YL, *et al*: Superoxide mediates sympathoexcitation in heart failure: roles of angiotensin II and NAD(P)H oxidase. *Circ Res* 2004; **95**: 937–944.
28. Campese VM, Ye S, Zhong H, Yanamadala V, Ye Z, Chiu J: Reactive oxygen species stimulate central and peripheral sympathetic nervous system activity. *Am J Physiol Heart Circ Physiol* 2004; **287**: H695–H703.
29. Shinomiya K, Mizushige K, Fukunaga M, *et al*: Antioxidant effect of a new calcium antagonist, azelnidipine, in cultured human arterial endothelial cells. *J Int Med Res* 2004; **32**: 170–175.
30. Yamagishi S, Inagaki Y, Nakamura K, Imaizumi T: Azelnidipine, a newly developed long-acting calcium antagonist, inhibits tumor necrosis factor-alpha-induced interleukin-8 expression in endothelial cells through its anti-oxidative properties. *J Cardiovasc Pharmacol* 2004; **43**: 724–730.