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Cilnidipine as an agent to lower blood pressure without sympathetic nervous activation as demonstrated by iodine-123 metaiodobenzylguanidine imaging in rat hearts

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Background: Administration of short-acting antihypertensive agents to patients with ischemic heart disease results in increased sympathetic nervous activity and is associated with worsened outcomes. Cilnidipine is an agent which blocks not only L-type calcium channels at the smooth muscle in the artery, but also N-type calcium channels at the presynaptic terminal. The goal of the present study was to determine the effect of cilnidipine on sympathetic nervous activity as on agent which blocks both L-type and N-type calcium channels at the presynaptic terminal, on sympathetic nervous activity in an experimental rat model using iodine-123 metaiodobenzylguanidine (MIBG) myocardial imaging. ***Methods:*** Fourteen-week-old Wistar-Kyoto rats were divided into 3 separate groups: CTR group (control: distilled water administered), Nif group (nifedipine administered), or Cil group (cilnidipine administered). Agents were administered via a stomach tube, followed by injection of MIBG via the femoral vein. Systolic blood pressure (SBP) and heart rate (HR) were measured by tail-cuff plethysmography just prior to administration of antihypertensive drugs and 150 minutes later. Initial imaging (Ce) and delayed imaging (Cd) were defined as the sum of density counts in the region of interest created by adjusting to myocardial edge, and were corrected for both physical decay and weight. The myocardial washout rate (WR) was defined as the percent change in the count density from the initial to delayed images. ***Results:*** Significant decreases in SBP were seen in the Nif group (from 132 ± 3 mmHg to 85 ± 5 mmHg, $p < 0.0001$) and the Cil group (from 128 ± 4 mmHg to 92 ± 7 mmHg, $p = 0.0008$), whereas no significant change in SBP was noted in the CTR group (from 123 ± 5 mmHg to 127 ± 3 mmHg). HR significantly increased in the Nif group (from 290 ± 12/min to 378 ± 14/min, $p < 0.0001$) but not in the CTR (from 278 ± 3/min to 300 ± 6/min) or Cil (from 291 ± 6/min to 303 ± 5/min) groups. WR was significantly greater in the Nif group (64.7 ± 0.5%) when compared to the CTR (56.4 ± 1.2%, $p = 0.0031$) or the Cil (55.4 ± 2.2%, $p = 0.0016$) groups. ***Conclusion:*** In contrast to nifedipine, administration of cilnidipine did not result in increased myocardial sympathetic nervous activation.

Key words: MIBG, washout rate, N-type Ca channel blocker, myocardial sympathetic nervous activation

INTRODUCTION

INCREASED sympathetic nervous activity following administration of short-acting antihypertensive drugs to patients with ischemic heart disease is associated with a poor prognosis,[1–3] whereas use of long-acting calcium antagonists have beneficial effects on cardiovascular mortality and/or morbidity.[4] Studies have demonstrated

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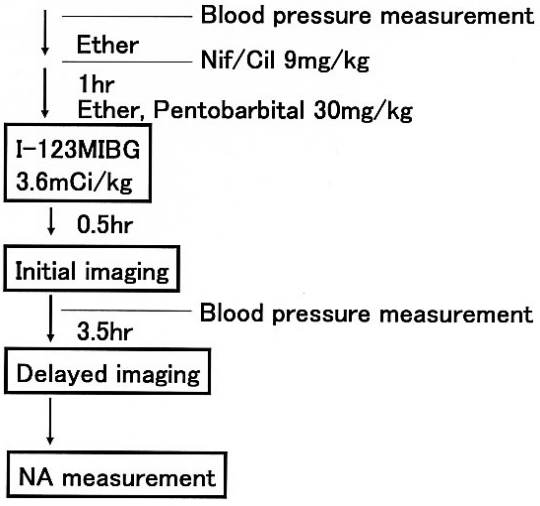

Fig. 1 Experimental schema. Nif: nifedipine, Cil: cilnidipine, NA: plasma noradrenaline.

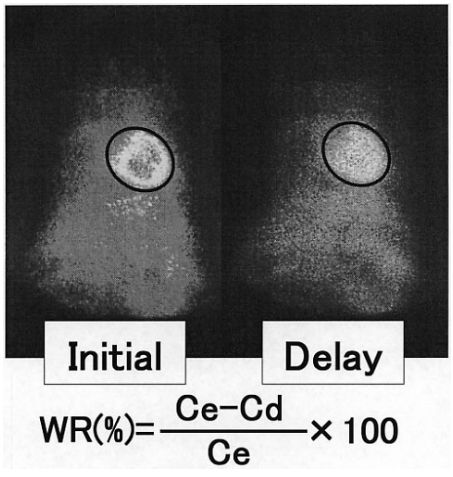

Fig. 2 MIBG imaging and calculation of WR. Initial imaging (Ce) and delayed imaging (Cd) were defined as the sum of density counts in the region of interest created adjust to myocardial edge.

that third-generation dihydropyridine-based calcium antagonists possess less sympathetic nervous activation effects than short-acting dihydropyridines.[5] Cilnidipine, an agent developed as a new class of calcium channel blockers, which blocks not only L-type calcium channels at the smooth muscle in the artery, but also N-type calcium channels at the presynaptic terminal of the sympathetic nerve.[6,7]

Measurement of the ratio of high-frequency component (HF) to low-frequency component (LF) in heart rate variability spectral analysis and iodine-123 metaiodobenzylguanidine (MIBG) imaging have been employed to assess cardiac sympathetic nervous activity and the severity of congestive heart failure.[8–11] However, only a few has been reported to assess changes in sympathetic

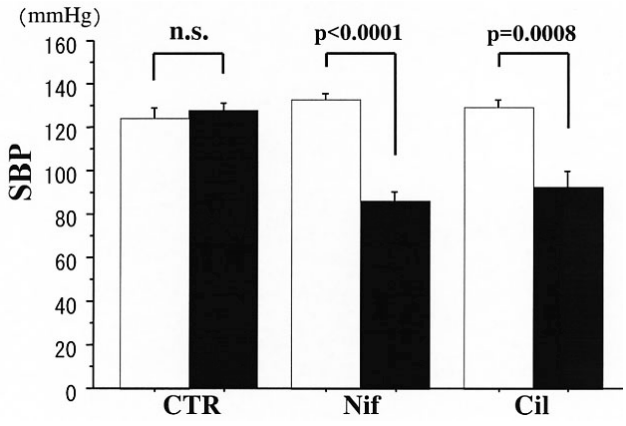

Fig. 3 Changes in SBP before and after administration of antihypertensive drugs. SBP: systolic blood pressure. Open bars: before administration of antihypertensive drugs, filled bars: after administration of antihypertensive drugs. CTR group (no antihypertensive drug), Nif group (nifedipine), Cil group (cilnidipine).

nervous activity following administration of various antihypertensive agents.[12,13] The goal of the present study was to determine the effect of cilnidipine on sympathetic nervous activity as an agent which blocks both L-type and N-type calcium channels at the presynaptic terminal, on sympathetic nervous activity in an experimental rat model using iodine-123 metaiodobenzylguanidine (MIBG) myocardial imaging.

METHODS

Anesthetized 14-week-old Wistar-Kyoto rats (Japan SLC, Inc.) were divided into 3 groups: CTR group (control: distilled water, n = 7), Nif group (nifedipine, 9 mg/kg, Bayer Yakuhin, Ltd., n = 6), and Cil group (cilnidipine, 9 mg/kg, Ajinomoto Co., Inc., Mochida Pharmaceutical Co., Ltd., n = 6). Anesthesia was performed first with ether (Wako Pure Chemical Industries, Ltd.) inhalation prior to drug administration and then with ether inhalation anesthesia and pentobarbital (30 mg/kg I.P., Dainabot Co., Ltd.) just prior to MIBG intravenous infusion.

Nifedipine and cilnidipine were dissolved in a 5% solution of Arabic Gum (Wako Pure Chemical Industries, Ltd.). Drugs and distilled water were administered via a stomach tube. One hour later, MIBG [133.2 MBq (3.6 mCi)/kg, specific activity 74 GBq (2000 mCi)/μg, Daiichi Radioisotope Laboratories, Ltd., Japan] was injected via the femoral vein. A 10-minute static acquisition was made every 30 minutes (initial) and 4 hours (delayed) after injection of MIBG in the anterior view. Cardiac images were acquired after each static acquisition, using a gamma camera (PRISM2000XP) (Picker International, Inc.) equipped with a pinhole collimator (Fig. 1). This pinhole collimator was used with an aperature 2 mm for high-resolution. Rats were restrained (limbs, upper teeth) in the

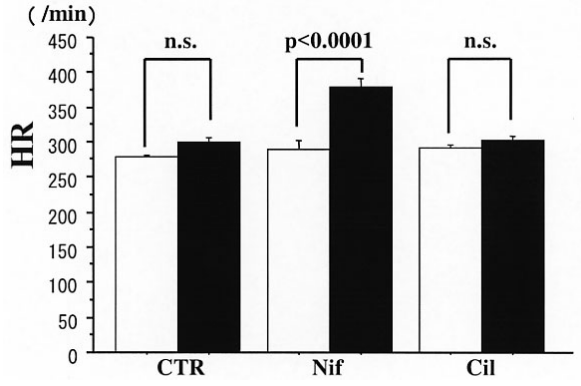

Fig. 4 Changes in HR before and after administration of antihypertensive drugs. HR: heart rate. Open bars: before administration of antihypertensive drugs, filled bars: after administration of antihypertensive drugs. CTR group (no antihypertensive drug), Nif group (nifedipine), Cil group (cilnidipine).

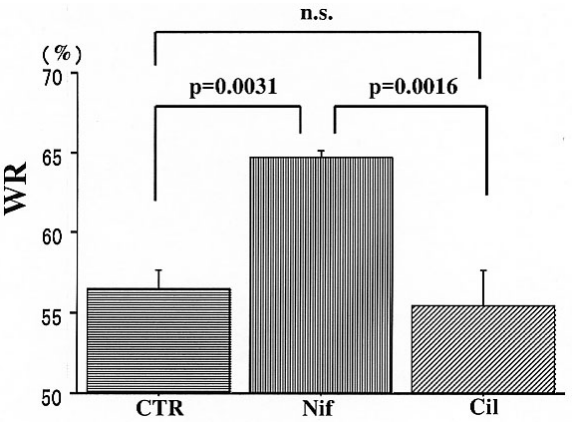

Fig. 5 Mean WR of each group. WR: washout rate. CTR group (no antihypertensive drug), Nif group (nifedipine), Cil group (cilnidipine).

supine position in order to fix the imaging point and prevent body motion artifact. Systolic blood pressure (SBP) and heart rate (HR) were measured by tail-cuff plethysmography (Softron Co. Ltd., Japan) just prior to administration of antihypertensive drugs and 150 minutes later. The room temperature was maintained at 24°C throughout the experiment.

After the delayed imaging, the rats were sacrified and blood sample was collected immediately. Blood samples were store at −80°C. The plasma noradrenaline (NA) concentraition was determined by a high-performance liquid chromatography.

Initial imaging (Ce) and delayed imaging (Cd) were defined as the sum of density counts in the region of interest created by adjusting to myocardial edge, and were corrected for both physical decay and weight. The myocardial washout rate (WR) was defined as the percent change in the count density between the initial and delayed images, and was calculated by following equation:

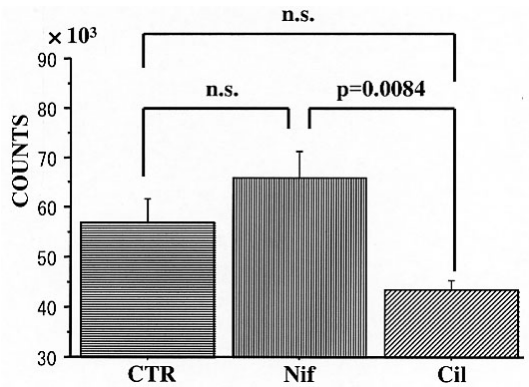

Fig. 6 Mean Ce of each group. Ce: initial imaging. CTR group (no antihypertensive drug), Nif group (nifedipine), Cil group (cilnidipine).

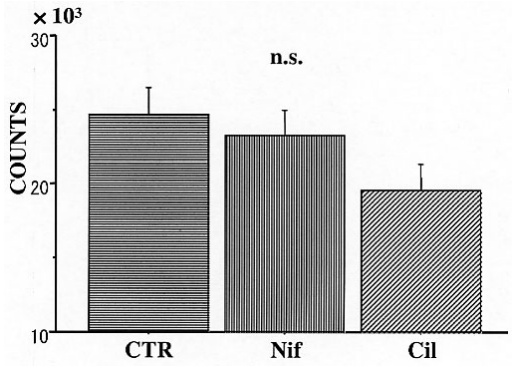

Fig. 7 Mean Cd of each group. Cd: delayed imaging. CTR group (no antihypertensive drug), Nif group (nifedipine), Cil group (cilnidipine).

$$WR\ (\%) = [(Ce - Cd)/Ce] \times 100 \text{ (Fig. 2).}$$

Statistical analysis

Data are expressed as mean ± S.E. Probability value <0.05 was considered significant. SBP and HR in the 3 groups (CTR, Nif, Cil) were compared using 2-way ANOVA before and after administration of antihypertensive drugs. Ce, Cd, WR and NA were compared among the 3 groups (CTR, Nif, Cil) using 1-way ANOVA. The difference in the SBP and HR, before and after administration of antihypertensive drugs (ΔSBP and ΔHR, respectively) were compared among the 3 groups (CTR, Nif, Cil) using 1-way ANOVA. Post-hoc test (Scheffe) was performed if significant differences were detected by ANOVA.

RESULTS

Significant decreases in SBP were seen in the Nif group (from 132 ± 3 mmHg to 85 ± 5 mmHg, $p < 0.0001$) and the Cil group (from 128 ± 4 mmHg to 92 ± 7 mmHg, $p = 0.0008$), whereas no significant change in SBP was noted

in the CTR group (from 123 ± 5 mmHg to 127 ± 3 mmHg). There was no significant difference in ΔSBP between the Nif (47 ± 6 mmHg) and the Cil (36 ± 8 mmHg) groups (Fig. 3). HR significantly increased in the Nif group (from 290 ± 12/min to 378 ± 14/min, $p < 0.0001$) but not in the CTR (from 278 ± 3/min to 300 ± 6/min) or Cil (from 291 ± 6/min to 303 ± 5/min) groups. ΔHR was significantly larger in the Nif group (89 ± 21/min) than in the CTR group (22 ± 6/min, $p = 0.0077$) or Cil group (12 ± 9/min, $p = 0.0035$) (Fig. 4).

WR was significantly greater in the Nif group (64.7 ± 0.5%) than in the CTR (56.4 ± 1.2%, $p = 0.0031$) or the Cil (55.4 ± 2.2%, $p = 0.0016$) groups (Fig. 5). Ce was significantly larger in the Nif group (65960 ± 5215) than in the Cil group (43330 ± 1882) ($p = 0.0084$) (Fig. 6). Cd did not differ among the groups (24628 ± 1737, 23235 ± 1696, and 19457 ± 1759 in the CTR, Nif, and Cil groups, respectively) (Fig. 7).

NA was significantly greater in the Nif group (442 ± 104 pg/m*l*) than in the CTR group (170 ± 42 pg/m*l*) ($p = 0.0336$), and there were no significant difference between the CTR group and the Cil group (281 ± 42 pg/m*l*) (Fig. 8).

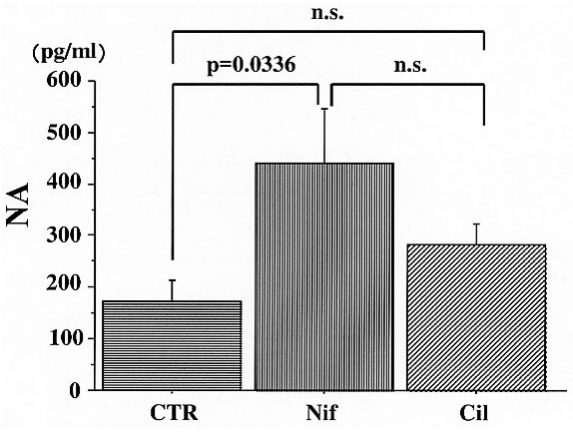

Fig. 8 Mean NA of each group. NA: plasma noradrenaline. CTR group (no antihypertensive drug), Nif group (nifedipine), Cil group (cilnidipine).

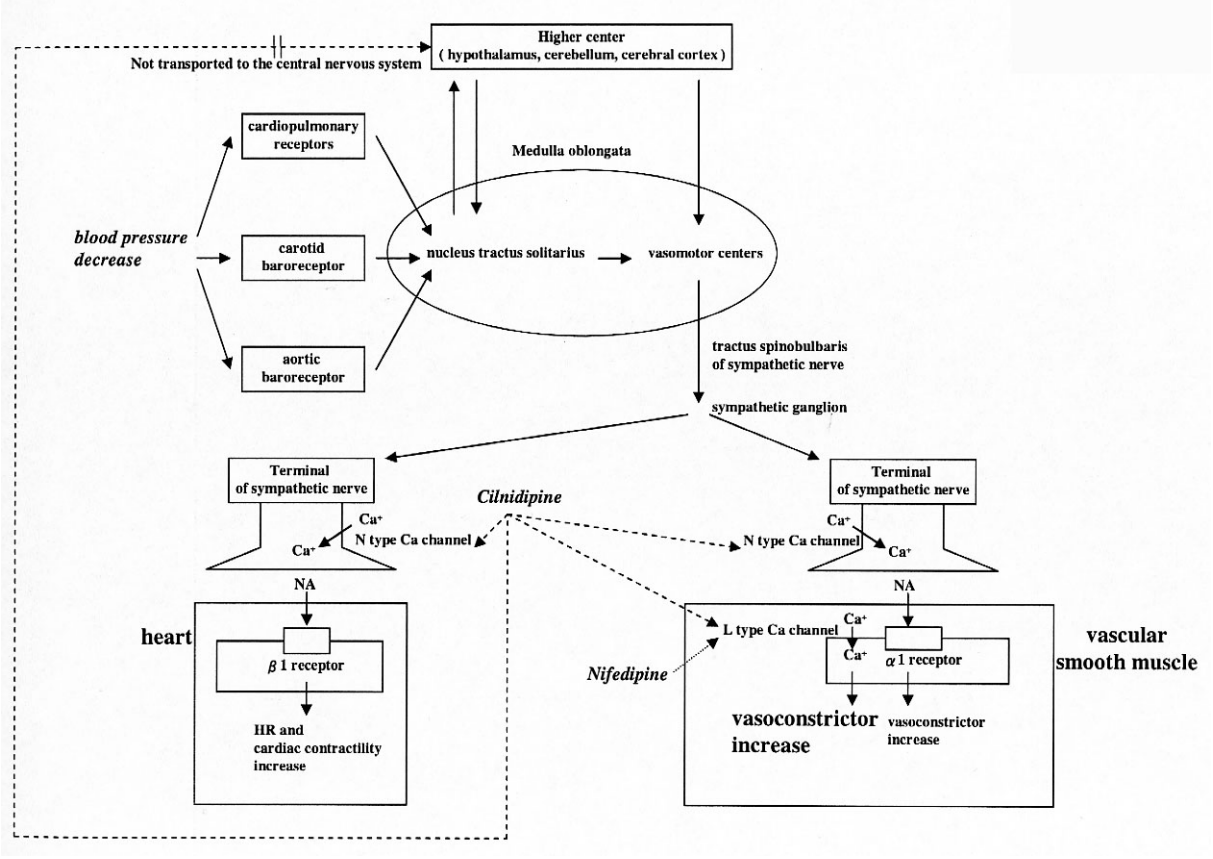

Fig. 9 Blood pressure regulation and sympathetic nervous activation. Baroreceptors distributed in the carotid and aorta perceive decreases in blood pressure and stimulate increases in blood pressure via the nucleus tractus solitarius and vasomotor centers in the medulla oblongata. Peripheral sympathetic outflow increases in response to this process with concomitant activation of N-type calcium channels and secretion of NA resulting in an increase in heart rate. Increases in HR, WR and NA were not seen after cilnidipine administration. This is likely secondary to antagonism of N- and L-type Ca channel in the cardiac sympathetic nervous terminal rather than a centrally mediated phenomenon, as cilnidipine does not pass through the blood brain barrier.

DISCUSSION

The use of short-acting calcium channel blockers in hypertensive patients with ischemic heart disease is associated with poor outcomes, and thus, the use of slow-onset, long-acting antihypertensive drugs is preferred. It is reported that in comparison with the placebo group, the blood pressure is lower in resting and exercise test on cilnidipine, but the heart rate does not change.[14]

Various methods have been employed to measure cardiac sympathetic nervous activation, including HF/LF ratio in heart rate variability spectral analysis and WR in MIBG imaging. However, HF/LF ratio is an imperfect measure of sympathetic nervous activation as LF is affected by both sympathetic and parasympathetic nerve activity.[15] In contrast, MIBG is an excellent measure of sympathetic nervous activity[16] and can yield data in the form of visual imaging or quantitative indices (heart-to-mediastinum ratio and WR).

While MIBG has been employed in patients with heart failure, there are few studies that describe its use in assessing changes in myocardial sympathetic nervous activity with various antihypertensive agents. The reports using MIBG in clinical hypertensive patients compared changes in sympathetic nervous activity after administration of enalapril, nitrendipine, amlodipine and cilnidipine[12,13] and demonstrated that WR decreased with enalapril, amlodipine and cilnidipine in comparison to nitrendipine. However, chronic effect of antihypertensive drugs was assessed in these reports, and there has been no report to assess acute effect with a similarly protocol.

In the present study, both HR and WR were increased in the Nif group, and SBP was reduced in the Nif and Cil groups compared to control (anesthesia only). While WR was higher in the Nif group when compared to the Cil group, ΔSBP did not differ between the two groups, suggesting that SBP decreased without sympathetic nervous activation following administration of cilnidipine. We speculate that the effect of cilnidipine is mediated through antagonism of the N-type Ca channel.

A key regulator of blood pressure is the carotid and aortic baroreflex. Baroreceptors distributed in the carotid and aorta perceive decreases in blood pressure and stimulate increases in blood pressure via the nucleus tractus solitarius and vasomotor centers in the medulla oblongata (Fig. 9). Peripheral sympathetic outflow increases in response to this process with concomitant activation of N-type calcium channels and secretion of NA resulting in an increase in heart rate. In the present study, increases in HR, WR and NA were not seen after cilnidipine administration. This is likely secondary to antagonism of N- and L-type Ca channel in the cardiac sympathetic nervous terminal rather than a centrally mediated phenomenon, as cilnidipine does not pass through the blood brain barrier.[17] Further, T-type calcium channels that are distributed in the sinus node were also unlikely to be affected by cilnidipine and nifedipine.

In the present study, NA levels were greater in the Nif group than in the control groups, while there was no significant difference between the Cil group and the control group. These data are consistent with previous studies performed in spontaneously hypertensive rats (SHR),[18] and other studies have demonstrated that there was no significant difference in NA levels when comparing 14-week-old WKY and SHR.[19,20]

Increased WR was associated with high Ce in the Nif group. We speculate that this may be due to: 1) increased reuptake of norepinephrine accompanied by increased exocytosis, and 2) a different influence on reuptake of norepinephrine by Ca channel blockers.

Study limitation

The action time of Nif was shorter than Cil, thus this difference might cause more sympathetic nervous activation in Nif group. We could not resolve the problem in the present study. Controversy would continue as to whether acute effect of the antihypertensive drugs in the present study brought chronic effect in the previous reports by Sakata, et al.

CONCLUSION

These data demonstrated that cilnidipine lowers blood pressure without the increase in sympathetic nervous activity that is seen with nifedipine. These observations may contribute to improvements in the clinical selection of antihypertensive agents in different patient populations.

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