Sympathoinhibition by Central and Peripheral Infusion of Nifedipine in Spontaneously Hypertensive Rats

Piotr P. Murzenok, Bing S. Huang, Frans H.H. Leenen

Abstract—The present study assessed whether central mechanisms may contribute to the hypotensive effect of the calcium channel blocker nifedipine. In conscious, spontaneously hypertensive rats (SHR) on a high-salt diet, hemodynamic (mean arterial pressure [MAP] and heart rate) and sympathetic (renal sympathetic nerve activity) responses to low, central, intracerebroventricular infusion rates (25 μg · kg⁻¹ · h⁻¹ for 2 hours) and peripheral intravenous rates (50 μg · kg⁻¹ · h⁻¹ for 3 hours and then 100 μg · kg⁻¹ · h⁻¹ for 2 hours) of nifedipine were evaluated. The distribution of nifedipine in the blood and tissues was assessed at the end of the infusions. Nifedipine significantly inhibited renal sympathetic nerve activity and lowered MAP in SHR beginning 30 minutes after the start of the intracerebroventricular infusion. The decrease of MAP by intravenous infusion began at 60 minutes and was more profound with 100 μg · kg⁻¹ · h⁻¹. Inhibition of sympathetic activity preceded and then paralleled the decrease in blood pressure; it occurred earlier with central (15 to 30 minutes) than with peripheral (30 to 60 minutes) infusion. Intravenous infusion resulted in concentrations of nifedipine in brain structures (brain stem, midbrain, and cortex) that were 30% to 40% of those in the heart, kidneys, and liver. From the hemodynamic and sympathetic responses and the distribution of nifedipine into the central nervous system, we conclude that the peripheral infusion of nifedipine at relatively low rates may evoke a hypotensive response in SHR, not only via peripheral mechanisms, but also through central mechanisms, which will lead to an inhibition of sympathetic outflow and, therefore, a lowering of blood pressure. (Hypertension. 2000;35:631-636.)

Key Words: nifedipine ■ infusion, intravenous ■ rats, inbred SHR ■ sympathetic nervous system ■ hemodynamics ■ pharmacokinetics

It is generally assumed that blocking the Ca²⁺ influx into the vascular smooth muscle cells causing vasodilation represents the main mechanism for the hypotensive effect of dihydropyridine calcium channel antagonists.¹ The decrease in blood pressure (BP) that occurs with rapid-acting dihydropyridines is usually accompanied by increases in heart rate (HR) and sympathetic activity due to arterial baroreflex activation.²–⁶ In contrast, low rates of infusion of nifedipine in spontaneously hypertensive rats (SHR),⁷ in dogs,⁸ and in humans⁹ or treatment with a slow-release formulation of nifedipine, nifedipine gastro-intestinal therapeutic system (GITS), in hypertensive patients¹⁰ do not cause sympathetic activation. This is generally assumed to relate to the resetting of arterial baroreceptors in parallel with the gradual fall in BP.⁵,¹¹–¹³

Dihydropyridines also have direct effects on the sympathetic nervous system. L-type voltage-gated calcium channels¹⁴ are the receptors for dihydropyridines¹⁵ throughout the rat central nervous system. Direct application of nifedipine on the dorsal surface of the brain stem decreased HR and BP in Wistar-Kyoto rats,¹⁶ whereas an intracerebroventricular (ICV) bolus injection of nifedipine decreased HR and BP in anesthetized SHR.¹⁷ Recently, we demonstrated that in conscious SHR, the ICV administration of nifedipine causes dose-dependent decreases in renal sympathetic nerve activity (RSNA), BP, and HR.⁶

It is presently unknown whether nifedipine, when administered peripherally, can act through central mechanisms. After intravenous (IV) injection, nifedipine has been detected in various organs, including the brain,¹⁸ with similar distributions in the white and gray matter.¹⁹ We hypothesized that during prolonged IV infusion at relatively low rates, sufficient nifedipine will penetrate into the central nervous system to inhibit sympathetic outflow and, thereby, lower BP. To test this hypothesis, SHR on a high-salt diet and that had an enhanced sympathetic tone²⁰,²¹ were studied using the following protocols: (1) BP, HR, and RSNA responses to central (ICV) infusion of nifedipine; (2) BP, HR, and RSNA responses during prolonged IV infusion of nifedipine at relatively low rates; and (3) the distribution of nifedipine in brain structures (brain stem, cortex, and midbrain) and other tissues (heart, liver, kidney, adrenal gland, and blood) at the end of the infusion.

Methods

Animals
Male SHR (Taconic Farms, Germantown, NY), aged 3.5 to 4 weeks on arrival, were used. The animals were housed in a facility with a
12-hour light/dark cycle and were allowed a 5-day acclimatization to normal rat chow and tap water. All animals were then put on a high-salt diet containing 8% NaCl for 4 to 6 weeks. All procedures were carried out according to the guidelines of the University of Ottawa Animal Committee for the use and care of laboratory animals. Three experimental protocols were used in different groups of rats.

**Protocol 1: BP and Tissue Distribution of Nifedipine After Intravenous Infusion**

Under halothane anesthesia, the left carotid artery and right jugular vein of 7.5- to 9.5-week-old rats were catheterized with polyethylene (PE-50) catheters in the afternoon of the day before hemodynamic assessment. For the ICV infusion of nifedipine, the carotid artery catheter was joined to a pressure transducer. The bridge output signal of the transducer was amplified (Transbridge TBM4, World Precision Instruments) and fed to an IBM-compatible computer equipped with a data acquisition program (Dataquest LabPro, Data Science International) that allowed on-line analysis of the pulsatile BP signal (sampling rate, 500 Hz) and data storage. The jugular vein catheter was joined to a Harvard pump for IV infusions. Two rats were monitored simultaneously. After a 20-minute rest, baseline mean arterial pressure (MAP) and HR were recorded for 30 minutes. Infusions of control solution to 1 animal and nifedipine solution to the other were then started. The nifedipine starting dose was 50 μg · kg⁻¹ · h⁻¹ at an infusion rate of 2 μL · min⁻¹ · 200 g⁻¹ for 3 hours. For the second 3 hours, the rate of infusion was increased to 100 μg · kg⁻¹ · h⁻¹ (infusion rate, 4 μL · min⁻¹ · 200 g⁻¹).

At the end of the infusion, blood and tissue samples were collected under yellow light. For blood collection, a solution consisting of 25% saline, 100 mg/mL; pH 7.4), and bidistilled water (0.045 mL) was used; 0.01 M of thiocyanate was used for the blank blood sample (2.02 mL). Blood samples were obtained from the carotid artery, placed in ice, processed in a cold room, and centrifuged for 7 minutes at 7000 rpm. The rats were then euthanized by an IV infusion of phenobarbital, and their organs were taken out (brain, heart, liver, kidney, and adrenal glands) and placed in dry ice. The tissue and plasma samples were stored at −20°C. The brain was divided into the cortex, brain stem, and the rest (midbrain).

**Protocol 2: RSNA and Hemodynamic Responses During Intravenous Infusion of Nifedipine**

In rats 8 to 9 weeks of age, early in the morning under halothane anesthesia, PE-50 polyethylene catheters were inserted into the left carotid artery and right jugular vein. Through a flank incision, a pair of silver electrodes (A-M System, Inc.) was placed around and fixed to the left renal nerve with silicone rubber (SilGi1 604, Wacker) to measure RSNA, as described in detail previously. At least 4 hours after recovery from the anesthesia, the intra-arterial catheter was connected to a pressure transducer, and BP and HR were recorded with a polygraph (Model 7E, Grass Instrument Co) and a Grass 7P44 tachograph, respectively. The electrodes were linked to a Grass PS11 bandpass amplifier. The jugular vein catheter was joined to the Harvard pump for IV infusions. One rat was studied per day. After a 20-minute rest, baseline MAP and HR were recorded for 30 minutes. Infusion of control solvent or nifedipine solution was then started. The starting dose of nifedipine was 50 μg · kg⁻¹ · h⁻¹ (infusion rate, 2 μL · min⁻¹ · 200 g⁻¹ for 3 hours). For the next 2 hours, the rate of infusion was increased to 100 μg · kg⁻¹ · h⁻¹ (4 μL · min⁻¹ · 200 g⁻¹).

**Protocol 3: RSNA and Hemodynamic Responses During ICV Infusion of Nifedipine**

Approximately 10 to 12 days before final assessments, under halothane anesthesia, a 23-gauge, stainless steel guide cannula was implanted and fixed to the skull of the remaining rats (coordinates: 0.4 mm posterior and 1.4 mm lateral to bregma; tip, 2.8 mm ventral to the dura) for the ICV infusion of nifedipine in the right ventricle. The cannulation of the carotid artery and jugular vein, the placement of electrodes on the renal nerve, and the registration of MAP, HR, and RSNA were performed in a manner similar to that used in protocol 2. Baseline MAP, HR, and RSNA were recorded for 30 minutes in the afternoon. Nifedipine was infused at 25 μg · kg⁻¹ · h⁻¹, with the rate of infusion of control and nifedipine solutions at 0.5 μL · min⁻¹ · 200 g⁻¹ for 2 hours. The ICV infusion was performed through an injection cannula placed into the guide cannula. One rat at a time was monitored continuously for MAP, HR, and RSNA. At the end of the infusion, blood samples were obtained. Subsequently, the rats were euthanized; background noise for RSNA was recorded 20 minutes later. At this point, brain samples were obtained for the determination of nifedipine levels, as described for protocol 1.

**Administration of Nifedipine**

The concentration of nifedipine in the stock solution was 0.1% in solvent; it consisted of 969 g of polyethylene-glycol 400 (Sigma), 60 g of glycerine (BDH Inc), and 100 g of water. The stock solution was prepared fresh every day and was diluted by saline. The control animal was infused with saline in the same concentration as in the final nifedipine solution. The infusion lines were covered with aluminum foil to protect the nifedipine from UV destruction. Rates of IV and ICV infusion of nifedipine were established in preliminary experiments with the goal of finding the rates of infusion that caused delayed and gradual decreases in BP to prevent/minimize baroreflex activation of the sympathetic nervous system. Thresholds for effects were in the range of 5 to 10 μg · kg⁻¹ · h⁻¹ for ICV infusions and in the range of 10 to 20 μg · kg⁻¹ · h⁻¹ for IV infusions.

**Determination of Nifedipine in Plasma and Tissues**

Plasma concentrations of nifedipine were determined using the gas chromatographic method with electron-capture detection, as described by Rönsch et al. with minor modifications. Rat plasma (250 μL) was mixed with 1 mL of toluene containing 10 μg/L nitrendipine as the internal standard and shaken at an ambient temperature for 20 minutes. After centrifuging at 4000 rpm for 10 minutes, 1 μL of the organic layer was injected into the gas chromatograph (HP 6890 Plus with μ-electron-capture detector and autosampler HP 7683, Hewlett Packard). The chromatographic separation was performed on a capillary column (DB-1, 30 m, 0.32 mm inner diameter, J&W Scientific), with helium as the carrier gas (2.5 mL/min) and argon/methane (95/5) as the make-up gas. The lower limit of quantitation was 1.0 μg/L. Calibration samples were prepared from blank rat plasma spiked with nifedipine. Interassay precision ranged from 7% to 4% at concentrations of 1.5 μg/L, 25 μg/L, and 80 μg/L, respectively. Accuracy ranged from 6% to 11%. Rat tissue samples (10 to 380 mg) were mixed with ~750 μL of 0.9% aqueous sodium chloride solution and homogenized with an Ultra-Turrax mixer (Janke & Kunkel). The volume of the homogenate was then adjusted to 1 mL with a 0.9% aqueous sodium chloride solution. Aliquots of 250 μL were mixed with 1 mL of toluene containing 10 μg/L nitrendipine as the internal standard and further worked up and processed as described for plasma samples. The lower limit of quantitation was ~2.6 ng/g tissue (depending on sample weight). Calibration samples were prepared from blank rat tissue homogenate spiked with nifedipine. Results were calculated in nanograms per grams of tissue. Interassay precision ranged from 9% to 7% at homogenate concentrations of 1.5 μg/L, 25 μg/L, and 80 μg/L, respectively. Accuracy ranged from 1% to 7%.

**Statistical Analysis**

Values are presented as mean±SE. Comparison of body weight, water intake, baseline MAP, and HR were determined by Student’s t-test. Responses of RSNA were expressed as percent changes from baseline values. One-way ANOVA for repeated measurements for changes of MAP, HR, and RSNA during infusion was performed. Statistic significance was defined as P<0.05.

**Results**

BP and HR During Prolonged IV Infusion of Nifedipine

The baseline MAP and HR were 162±6 and 167±4 mm Hg and 380±11 and 415±5 bpm in control (n=7) and nifed-
pine-treated (n=9) SHR, respectively. No substantial changes of MAP occurred in SHR relative to baseline levels during the infusion of the control solution (Figure 1). In nifedipine-treated SHR, a gradual decrease of MAP was observed beginning 60 minutes after the start of infusion at 50 μg·kg⁻¹·h⁻¹ (Figure 1). Significant decreases of MAP (15 to 18 mm Hg) compared with baseline and corresponding controls were noted at 120, 150, and 180 minutes after the start of infusion. Increasing the infusion rate to 100 μg·kg⁻¹·h⁻¹ resulted in a further (P<0.05) decrease of MAP, which was significant at all time intervals during the 3-hour infusion period. The decreases of MAP compared with baseline varied from 18 to 24 mm Hg.

In control SHR, no changes in HR occurred over the 6-hour period of control infusion. In nifedipine-treated animals, HR did not change significantly compared with baseline at both the 50 and 100 μg·kg⁻¹·h⁻¹ infusion rate (Figure 1). At some intervals, HR was significantly lower in comparison with the control group.

RSNA During Prolonged IV Infusion of Nifedipine
The baseline MAP and HR were 130±5 and 136±5 mm Hg and 431±23 and 432±12 bpm in control (n=7) and nifedipine-treated (n=7) SHR, respectively. The IV infusion of control solution was associated with small, nonsignificant increases in MAP (by 5 to 10 mm Hg) and RSNA (by 5% to 10%) (Figure 2). Prolonged IV infusion of nifedipine at 50 and 100 μg·kg⁻¹·h⁻¹ decreased RSNA and MAP without affecting HR (Figure 2). Significant decreases of both RSNA and MAP with nifedipine (50 μg·kg⁻¹·h⁻¹) started at 60 minutes. A decrease of RSNA (~19%) was noted at 30 minutes, whereas MAP was still similar in control and nifedipine-treated animals at this time point. In comparison with corresponding control values, the average decrease in MAP during the second and third hours at 50 μg·kg⁻¹·h⁻¹ was 15 mm Hg. During the infusion at 100 μg·kg⁻¹·h⁻¹, BP decreased by 20 to 25 mm Hg. The decrease of RSNA compared with controls did not differ significantly with rates of 50 or 100 μg·kg⁻¹·h⁻¹ (31% to 40% and 35% to 42%, respectively).

RSNA and BP During ICV Infusion of Nifedipine
The baseline MAP in these groups of control (n=5) and nifedipine-treated SHR (n=5) was 134±3 and 148±5 mm Hg (P<0.05), respectively. ICV infusion of vehicle alone did not change MAP, but it slightly increased RSNA and HR (Figure 3). ICV administration of nifedipine resulted in decreases in MAP, RSNA, and HR. Significant changes of MAP and RSNA were noted 30 minutes after the start of the ICV infusion of nifedipine. In comparison with the baseline at 30, 60, 90, and 120 minutes,
Distribution of Nifedipine in SHR Plasma and Tissues after IV and ICV Infusions

<table>
<thead>
<tr>
<th>Samples</th>
<th>IV Infusion (n=6)</th>
<th>ICV Infusion (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>17±2</td>
<td>&lt;Detection</td>
</tr>
<tr>
<td>Midbrain</td>
<td>18±2</td>
<td>&lt;Detection</td>
</tr>
<tr>
<td>Brain stem</td>
<td>23±3</td>
<td>&lt;Detection</td>
</tr>
<tr>
<td>Heart</td>
<td>47±8</td>
<td>Not done</td>
</tr>
<tr>
<td>Liver</td>
<td>55±7</td>
<td>Not done</td>
</tr>
<tr>
<td>Kidney</td>
<td>60±6</td>
<td>Not done</td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>153±19</td>
<td>Not done</td>
</tr>
<tr>
<td>Plasma</td>
<td>87±8</td>
<td>8±1</td>
</tr>
</tbody>
</table>

Nifedipine measurements are ng/g of tissue or μg/L of plasma.

a gradual decrease occurred in MAP by 14±2, 16±2, 19±3, and 21±3 mm Hg and in RSNA by 32±5%, 34±5%, 42±11%, and 43±6%, respectively. ICV administration of nifedipine caused small decreases (P<0.05) in HR beginning 30 minutes after the start of the infusion.

Comparison of BP and sympathetic responses by ICV and IV infusions showed 2 differences (Figure 2 versus 3). First, the changes in BP and RSNA started earlier with the central compared with the peripheral administration of nifedipine. Second, the inhibition of sympathetic activity preceded the decrease of BP during IV infusion, whereas the 2 parameters decreased in parallel with ICV infusion.

Distribution of Nifedipine After IV and ICV Infusion

The tissue distribution of nifedipine in SHR after IV infusion at 50 μg · kg⁻¹ · h⁻¹ for 3 hours and then 100 μg · kg⁻¹ · h⁻¹ for 3 hours was as follows: adrenal glands > kidney > liver > heart > brain stem > midbrain > cortex (Table). The nifedipine concentration in plasma reached 87±8 μg/L. The accumulation of nifedipine among the tissues investigated was ≈3 times higher in the adrenal glands than in the kidney, liver, and heart and ≈8 times higher than in the brain stem, midbrain, and cortex. The accumulation of nifedipine in the brain stem was significantly higher than in the cortex and midbrain. ICV infusion (25 μg · kg⁻¹ · h⁻¹ for 2 hours) resulted in detectable levels of nifedipine in the plasma but not in the brain structures: nifedipine in the brain stem, midbrain, and cortex was no longer detectable in most animals 20 minutes after the termination of the infusion.

Discussion

In the present study, in conscious SHR, the following new findings were obtained: (1) IV infusion of nifedipine results in clear concentrations of nifedipine in the brain; and (2) similar to direct ICV infusion, a low rate of IV infusion of nifedipine evokes gradual decreases of both BP and RSNA.

After a single IV injection, nifedipine enters the brain, with similar distributions in the white and gray matter.18,19 In rats, radiolabeled nifedipine (1 mg/kg IV injection) reached the brain and hypophysis, but to a lesser extent than the liver, kidney, and lungs.18 In our experiments, prolonged IV infusion resulted in substantial concentrations of nifedipine in brain structures such as the cortex, brain stem, and midbrain. Among the brain structures investigated, nifedipine accumulated more in the brain stem than in the cortex and midbrain. Levels of nifedipine in the brain stem were ≈30% to 40% of those observed in the kidney, liver, and heart.

After a single bolus administration, the brain concentration of nifedipine was 3 to 4 times less than that in the liver and kidney after 2 minutes, but 10 to 20 times less than that in those structures after 8 hours.18 In the present study, very low concentrations of nifedipine were found in the brain 20 minutes after discontinuing the ICV infusion of nifedipine. In contrast, plasma concentrations of nifedipine at the end of the ICV infusion were detectable. It seems that nifedipine readily penetrates the brain-blood barrier and that an accumulation of nifedipine in the brain does occur with prolonged IV infusion and steady-state concentrations in the plasma in the 50 to 100 μg/L range. However, the low brain concentrations seen 20 minutes after discontinuing the ICV infusion, along with the above-stated results after a single IV injection of radiolabeled nifedipine,18 also suggest the rapid elimination of nifedipine from the brain.

With the direct registration of RSNA, we demonstrated the significant new finding that in conscious SHR, decreases of BP induced by the IV infusion of nifedipine are associated with and seem to be the result of the inhibition of peripheral sympathetic nerve activity. First, during the IV infusion of nifedipine, decreases of RSNA tended to precede the fall in BP. Second, the decrease of BP clearly occurred later after the start of IV than after ICV infusion (60 and 30 minutes after the start, respectively). Third, the lower dose of ICV-infused nifedipine (25 μg · kg⁻¹ · h⁻¹) induced similar falls in BP and an even more profound inhibition of RSNA as IV infusion at 50 and 100 μg · kg⁻¹ · h⁻¹. Moreover, the concentration of nifedipine in plasma by ICV infusion was 10 times less than that with IV infusion. We can, therefore, rule out the peripheral effects of centrally administered nifedipine. Altogether, these findings suggest that the BP responses observed during IV infusion of nifedipine at low rates may be due to the gradual accumulation of the calcium channel blocker in central nervous system structures.

The present study clearly indicates that a central sympathoinhibitory effect can occur after the peripheral administration of a dihydropyridine. Consistent with this finding, in SHR, long-term oral treatment with nisoldipine significantly decreased BP associated with decreased cardiac sympathetic activity, as assessed by cardiac norepinephrine turnover rate.20 Similarly in SHR, long-term treatment with amlodipine or manidipine lowered plasma norepinephrine, whereas hydralazine caused a further increase.27 The extent of such central effects with peripheral (oral or IV infusion) treatment is likely related to the degree of lipophilicity, which determines the speed and extent of the crossing of the blood-brain barrier. However, in humans13 and rats,6 after the peripheral administration of fast-acting dihydropyridines, rapid decreases in BP by arterial vasodilation are associated with increases in sympathetic activity, suggesting that excitatory baroreflex-mediated responses in sympathetic activity prevail. A lack of sympathoinhibition may also be due to the short half-life of such dihydropyridines, ie, the latter may be
inactivated before reaching the brain tissue in sufficiently large amounts. Thus, differences in both lipophilicity as well as pharmacokinetics may determine the extent of the central effects of specific dihydropyridines. Finally, the dose may also be relevant. For example, at low doses of nifedipine GITS or other dihydropyridines, such as lacidipine, central effects may prevail, whereas at higher doses, peripheral arterial vasodilation and, possibly, sympathoexcitation may become more prominent.28

Along with its central effects, nifedipine may influence the sympathoadrenal compartment of the autonomic nervous system. The nifedipine concentration in the adrenal glands after prolonged IV infusion was the highest among the tissues investigated. Whether such selective accumulation in the adrenal glands is characteristic for SHR or also occurs in other forms of hypertension is, at yet, unknown. Dihydropyridine receptors in adrenal medulla membranes associated with L-type Ca2+ channels have been identified.29 The K+-evoked release of epinephrine and norepinephrine in bovine adrenal medulla are preferentially controlled by Q- and L-type Ca2+ channels, respectively.30 Nifedipine, acting through L-type Ca2+ channels, inhibits catecholamine uptake into storage vesicles31,32 and evokes an attenuation of the net increase in adrenal venous epinephrine concentration during splanchic nerve stimulation by IV infusion in dogs.33 Therefore, a selective accumulation of nifedipine in the adrenal glands may represent another sympatholytic mechanism during prolonged IV infusion.

In conclusion, our findings indicate that nifedipine, administered peripherally, may influence BP not only via peripheral mechanisms, by inhibiting the contraction of vascular smooth muscle cells and perhaps adrenal medulla functions, but also centrally, by blocking the activity of the L-type voltage-gated calcium channels of neurons in autonomic regulatory centers, leading to the inhibition of sympathetic activity and the lowering of BP. At low plasma concentrations, central sympathoinhibitory effects may represent the predominant antihypertensive mechanism, whereas at higher plasma concentrations, vascular smooth muscle cell relaxation contributes as well, and it may prevail with rapid-acting and/or hydrophilic dihydropyridines.

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References
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